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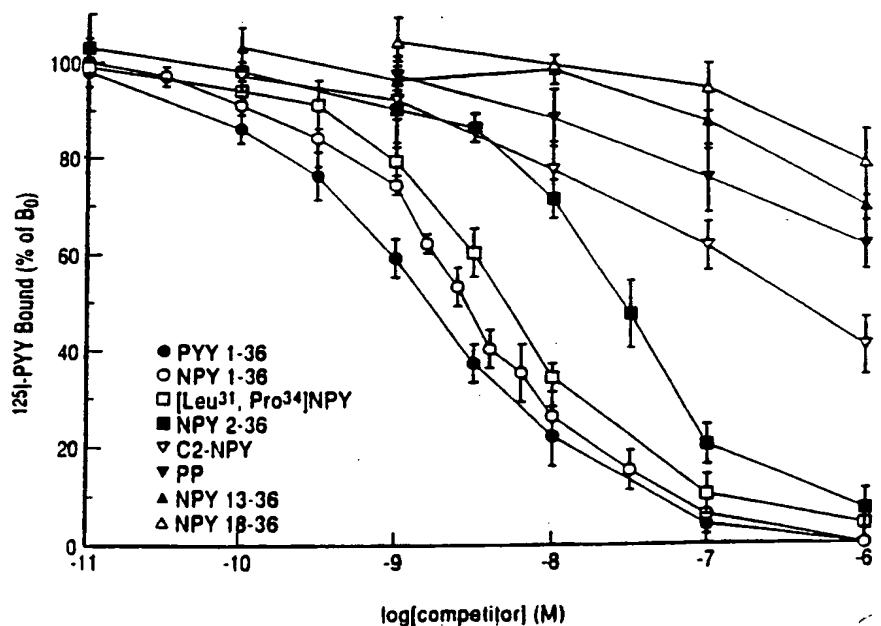
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>5</sup> : C07H 21/04, C12N 1/21, 5/00 C12P 21/00		A1	(11) International Publication Number: WO 93/24515 (43) International Publication Date: 9 December 1993 (09.12.93)
(21) International Application Number: PCT/US93/05039 (22) International Filing Date: 27 May 1993 (27.05.93)  (30) Priority data: 891.453 29 May 1992 (29.05.92) US  (71) Applicant (for all designated States except US): CORNELL RESEARCH FOUNDATION, INC. [US/US]; 20 Thornwood Drive, Ithaca, NY 14850 (US). (72) Inventor: and (75) Inventor/Applicant (for US only): WAHLESTEDT, Claes [SE/US]; 131 East 66th Street, New York, NY 10021 (US). (74) Agent: YAHWAK, George, M.; Yahwak & Associates, 25 Skytop Drive, Trumbull, CT 06611 (US).		(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  Published With international search report.  <div style="border: 1px solid black; padding: 5px; width: fit-content; margin: 10px auto;">         9558072158          44743-A-PCT-890       </div>	

(54) Title: HUMAN NEUROPEPTIDE Y/PEPTIDE YY RECEPTOR OF THE Y1-TYPE AND ANTISENSE OLIGONUCLEOTIDES THERETO WHICH INHIBIT VASOCONSTRICTION



(57) Abstract

The present invention is directed to the cloning, identification and uses of the receptor. The isolated DNA clone is expressed in COS1 cells for ligand binding coriple for the development of an inhibitor of the contractile responses of neuropepti antisense oligodeoxynucleotide complementary to the human Y-Y1 receptor mRNA

Jonathan A. Bard, et al.  
U.S. Serial No.: 08/495,695  
Filed: January 13, 1997  
Exhibit 10

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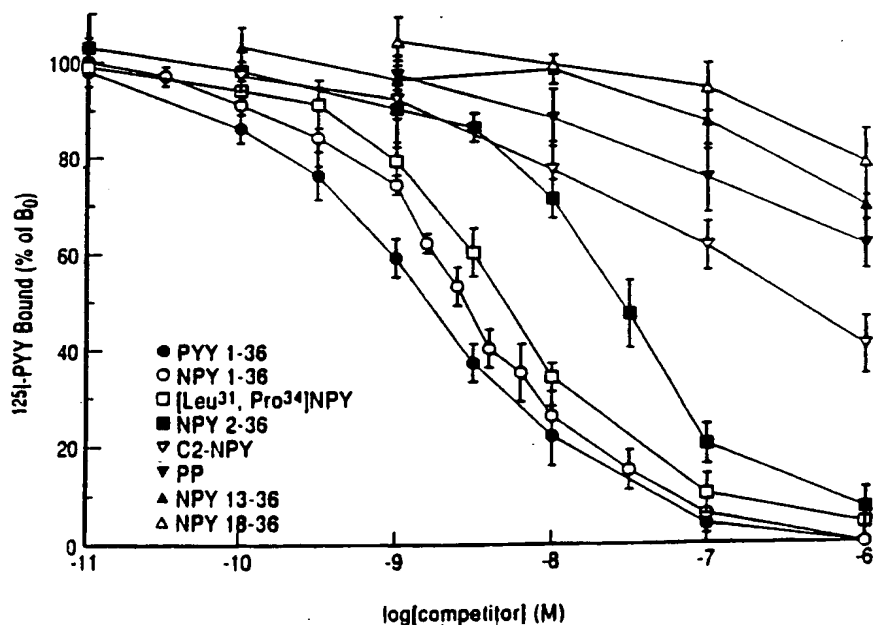
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(72) Inventor; and			
(75) Inventor/Applicant (for US only): WAHLESTEDT, Claes [SE/US]; 131 East 66th Street, New York, NY 10021 (US).			
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## (57) Abstract

The present invention is directed to the cloning, identification and uses of the human Y-1 type neuropeptide Y/peptide YY receptor. The isolated DNA clone is expressed in COS1 cells for ligand binding competition assay. Also described is a new principle for the development of an inhibitor of the contractile responses of neuropeptide Y in human blood vessels by the use of an antisense oligodeoxynucleotide complementary to the human Y-Y1 receptor mRNA.

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**HUMAN NEUROPEPTIDE Y/PEPTIDE YY RECEPTOR  
OF THE Y1-TYPE AND ANTISENSE OLIGONUCLEOTIDES THERETO  
WHICH INHIBIT VASOCONSTRICTION**

Partial funding of the research leading to the invention described  
5 herein was provided by the National Institute of Drug Abuse and the  
National Heart and Lung Institute. Accordingly the federal government  
has certain rights to this invention under 35 U.S.C. § 200 et seq.

Under United States patent practise, this application for Letters  
Patent is a Continuation-in-Part application of my earlier United  
10 States Patent Application 891,453, filed May 29th 1992.

Neuropeptide Y (NPY) and peptide YY (PYY) are structurally related  
peptides that primarily function as neurotransmitter and  
gastrointestinal hormone, respectively. Previous functional and binding  
data have indicated the existence of at least three distinct receptor  
15 types, Y1, Y2, and Y3, for NPY and/or PYY in mammals. We describe here  
a human Y1 cDNA clone, hY1-5, isolated from a fetal brain library. The  
human Y-1 receptor consists of 384 amino acids and has seven putative  
transmembrane (TM) domains like other members of the G-protein-  
coupled superfamily of receptors. In the region spanning the TM  
20 domains, the Y-1 receptor displays 29% sequence identity to human  
tachykinin receptors, but it only shows 21% and 23% homology with  
proposed bovine (LCR1) and Drosophila (PR4) NPY-receptor clones,  
respectively. Northern blot analysis of a human neuroblastoma cell  
line, SK-N-MC, previously used by many investigators as a model  
25 system for studies on the Y-1 receptor, revealed a single 3.5 kb mRNA  
species. Reverse transcriptase analysis (RT-PCR) indicated expression  
also in human cultured vascular smooth muscle cells, supporting the  
view that the Y1-receptor is associated with NPY/PYY-evoked  
vasoconstriction. When expressed in COS1 cells, hY1-5 conferred  
30 specific <sup>125</sup>I-PYY binding sites with displacement patterns  
characteristic of the Y1-receptor, i.e. PYY ≥ NPY ≥ [Leu<sup>31</sup>, Pro<sup>34</sup>]NPY >>  
NPY2-36 > C2NPY > pancreatic polypeptide > NPY13-36 > NPY18-36.  
Moreover, in the Y1-receptor transfected COS1 cells, but not in type 1  
angiotensin II receptor transfected control cells, NPY and PYY

accelerated  $^{45}\text{Ca}^{2+}$  influx and inhibited forskolin-stimulated cAMP accumulation, both phenomena being characteristic of the mammalian Y-1 receptor.

Neuropeptide Y (NPY) is a tyrosine-rich 36-amino acid peptide  
5 with a carboxyterminal amide which displays a remarkable degree of structural conservation in evolution. It is one of the most abundant and widely distributed neuropeptides within the central nervous system and belongs to a peptide family that also includes peptide YY (PYY) and pancreatic polypeptide (PP). Mammalian NPY and PYY show 70%  
10 sequence identity while PP is 50% homologous to NPY and PYY. NPY is widely distributed in the brain, notably in "limbic" regions, and the peripheral nervous system, and is often co-localized with norepinephrine, e.g. in sympathetic perivascular nerve fibers, supplying the cardiovascular system [see Trends in Pharmac. Sci. 8:231 (1987)].  
15 In the brain many effects, including stimulation of appetite, anxiolysis/sedation and modulation of pituitary hormone release, have been attributed to NPY/PYY. Among the many peripheral actions of NPY, it has been suggested to be involved in a large number of neuroendocrine functions, stress responses, circadian rhythms, central autonomic  
20 functions, eating and drinking behavior, and sexual and motor behavior; most attention has been given to its vasoconstrictor effects. In addition, it is also possible that NPY is related to various neurological and psychiatric illnesses such as Huntington's Chorea, Alzheimer's disease, and major depressive illness. However, in the absence of  
25 specific receptor antagonists, functional studies and receptor characterizations have been difficult to perform.

Based on functional and binding data obtained from studies of various organs and cell types, it has previously been suggested that NPY/PYY receptors are heterogeneous [see Ann. NY Acad. Sci. 611:7  
30 (1990); Regul. Pept. 12:317 (1986); and Life Sci. 50: PL7 (1992)] and the nomenclature "Y1-, Y2- and Y3-receptor type" was introduced to encompass this heterologous nature. The Y1-receptor binds NPY and PYY with similar affinity, as well as the synthetic analog [Pro<sup>34</sup>]NPY and analogs thereof, but C-terminal fragments of NPY and PYY have been

shown to bind poorly. In contrast, while the Y2-receptor also binds NPY and PYY with high affinity, the C-terminal fragments, e.g. NPY13-36, as well as a centrally truncated analog C2NPY [see Ann. NY Acad Sci. 611:35 (1990)], are only slightly less potent than the intact peptides at this receptor type. More recently, data from several laboratories [see Trends Pharmacol. Sci. 12:389 (1991)] have indicated the existence of a Y3-type of receptor, whose main characteristic is that PYY shows markedly lower affinity than NPY.

In order to address the structural and functional relationships of the NPY/PYY receptors the present invention pursued the isolation of receptor DNA clones using several strategies. These strategies led to the cloning of a putative human Y1-receptor cDNA clone. This clone, hY1-5, appears to be a human homolog of a previously published rat "orphan" receptor, FC5 [see FEBS Lett. 271:81 (1990)]. The latter rat clone had appeared relevant to the present invention because its expression pattern, as studied by *in situ* hybridization, was reminiscent of that of the Y1-receptor protein, as shown by receptor autoradiography. Thus, a polymerase chain reaction (PCR) product was generated corresponding to the rat "orphan" receptor. Using this, homologous human cDNA clones were isolated.

It is, accordingly, one aspect of the present disclosure is to present functional evidence identifying one such clone as a human NPY/PYY receptor of the Y1-type.

With the successful cloning of the NPY-Y1 receptor as described herein for the first time, and on the basis of the predicted mRNA sequence, another aspect of the present invention is to describe an 18-base antisense oligodeoxynucleotide sequence that corresponds to a coding region near the human Y1-receptor amino-terminus.

Still a further aspect of the present disclosure is to describe the inhibitory effect of treatment with neuropeptide Y-Y1 receptor antisense oligodeoxynucleotide on the contractile response to NPY of human arteries and veins.

The following figures, disclosure and examples are provided to allow one to receive a more complete understanding of the present

invention. These examples are not intended nor provided to limit the scope of the present invention in any manner, and it would be improper for one to interpret them as doing so (for example although Example VI depicts only a single antisense sequence, hY1-AS, this sequence is to be considered as merely a specific example of a class of antisense sequences which have similar capabilities of affecting the NPY-evoked contractile response of blood vessels as described herein).

With reference to the accompanying figures,

Figure 1 is a side-by-side comparison of Northern and Southern hybridizations;

Figure 2 depicts ligand competition for  $^{125}\text{I}$ -PYY binding in hY1-5 (Y1-receptor) transfected COS1 cell membranes;

Figure 3 depicts the contractile effect of NPY on human subcutaneous arteries; and

Figure 4 depicts the contractile effect of NPY on human veins.

More specifically, as will be described in detail below, Figure 1 depicts the Northern blot of human neuroblastoma cell lines probed with a human Y1 fragment, each lane containing 15  $\mu\text{g}$  of total RNA. Figure 1 also depicts the Southern blot of human genomic DNA under conditions of high stringency, with each lane containing 10  $\mu\text{g}$  of genomic DNA.

With regard to Figure 2, the competition data are presented as a percentage of specific binding in the absence of competitor wherein each point is the mean  $\pm$  SEM of two triplicate experiments. The concentration of  $^{125}\text{I}$ -PYY was 0.1nM. Each tube contained membranes (crude particulate fractions) from  $2 \times 10^6$  COS1 cells. Non-specific binding was defined as binding in the presence of 1  $\mu\text{M}$  unlabeled NPY.

With respect to Figures 3 and 4, as described above, these figures depict the contractile effect of NPY on human subcutaneous (Figure 3) arteries (diameter of  $0.41 \pm 0.03$  mm) and (Figure 4) veins (diameter of  $0.43 \pm 0.03$  mm) expressed as a percent of the contraction induced by 60 mM KCl. To obtain the data depicted, all vessels were incubated at 37° C for 48 hours. The symbols indicate treatment with (•) 1  $\mu\text{M}$  antisense, (O) 1  $\mu\text{M}$  sense, (I) 1  $\mu\text{M}$  mismatch or (/) control, i.e., no



oligodeoxynucleotide. As shown, the contractile response to NPY was markedly reduced after antisense treatment. Values represent the mean  $\pm$  SEM; n represents 8-10, except for the mismatch where n represents 3; \* represents  $p < 0.05$ ; and \*\* represents  $p < 0.01$  between sense and antisense (paired Wilcoxon signed rank test).

#### EXAMPLE I

##### Screening of a Human Fetal Brain cDNA Library

##### 1. Human Fetal Brain cDNA Library:

The lambda ZAPII cDNA library (Stratagene) was made from mRNA of a human female fetal (17-18 week gestation) brain, using both oligo (dT) and random-sequence primers.

##### 2. Transfer to Nylon Membranes:

After titrating the fetal brain lambda ZAP cDNA library ( $10^9$  pfu/ml), aliquots containing 50,000 phage particles were mixed with 0.2 ml of the host bacteria (XL1), which were then infected by incubating the mixture for 20 minutes at 37°C. In total, 200,000 clones were screened. Next, 6.5 ml top agarose (0.6%, at about 50°C) were added to the aliquots and poured onto 150 mm agar plates warmed to 37°C. The plates were incubated at 37°C for about 6-8 hours or until the plaques were confluent. The plates were cooled at 4°C for 2 hours before applying nylon filters. Biotrans nylon membrane (ICN) were placed onto the surface of the top agar, and markings were made with a syringe needle containing radioactive India ink for identification and orientation purposes. The filters were submerged in denaturing solution (1.5 M NaCl and 0.5 M NaOH) for 2 min and in neutralizing solution (1.5 M NaCl, 0.5 M Tris-HCl, pH 8.0) for 5 minutes, and then rinsed in 2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0). The filters were dried on Whatman 3MM paper and DNA was fixed to the filters by either using a UV Stratalinker 1800 (Stratagene) or baking at 80°C for 2 hours in a vacuum oven.

##### 3. Probe Preparation:

A 500-bp PCR product, corresponding to part of the coding region (547-1047) of the rat orphan receptor was used to screen the human

fetal brain cDNA library. This probe was generated using the following PCR conditions: 5 min at 95°C for 1 cycle, then 1 min at 93°C, 1 min at 45°C and 2 min at 72°C for 35 cycles, with the fetal brain cDNA library as template, and a 23-mer forward primer

- 5 (TTCCAAAATGTATCACTTGCGGC, positions 547-569) and a 25-mer reverse primer (TAGTCTCGTAGTCCGTCCGTCTCGAG, positions 1023-1047). Both primers were synthesized on a Biosearch Cyclone DNA Synthesizer. The PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl<sub>2</sub>, 1.6 mM dNTPs (US  
10 Biochemical), 50 pmol of forward and reverse primers, and 1 unit Taq DNA Polymerase in a 100 µl reaction volume.

#### 4. Probe Labeling:

- The human fetal brain probe was labeled using a random primed DNA labeling kit (Boehringer Mannheim Biochemical), following the  
15 manufacturer's instructions. Approximately 25 ng of the human fetal brain PCR product was heat denatured (10 minutes at 95°C), and the following components were added: dATP, dGTP, dTTP mixture (all 0.5 mmol/l in Tris buffer); reaction mixture (10X buffer with random hexamer primers); 50 µCi [alpha-<sup>32</sup>P] dCTP, 3000 Ci/mmol; and 1 unit  
20 Klenow enzyme. This mixture was incubated for 30 minutes at 37°C, and heated at 65°C for 10 minutes to stop the reaction. The probe was then purified by Sephadex G-50 Sin Columns to remove non-incorporated deoxyribonucleotide triphosphates. The Pharmacia Oligolabelling Kit was also used to label the human fetal brain probe.

#### 25 5. Hybridization Conditions:

- The filters were prehybridized for 2 hours at 42°C in 25% formamide, 1 M NaCl, 10% dextran sulfate, 5X Denhardt's solution and 1% SDS. The hybridization was carried out in the same solution with the addition of the <sup>32</sup>P-labeled human fetal brain probe (300 µl volume,  
30 200-300 cps/µl) at 42°C for 16 hours. The filters were then washed twice for 5 minutes at room temperature in 2X SSC, 0.2% SDS and twice at 42°C for 30 minutes in 2X SSC, 0.5% SDS. The nylon membranes were exposed to XAR-5 (Kodak) film at -70°C for 24-72 hours.

#### 6. Secondary Screening:

The positive plaques were removed from the plates and placed in SM buffer ((0.1 M NaCl, 0.01 M MgSO<sub>4</sub>, 50 mM Tris-HCl (pH 7.5), and 0.01% gelatin)). These plaques were diluted and titered with XL1 cells to yield about 10 plaques for the first set and 100 plaques for the second set on 100 mm agar plates. As before, the plates were incubated at 37°C overnight and transferred to nylon membranes as previously described. The same prehybridization/hybridization conditions in the initial screening were also used for the secondary screening. Positive clones were chosen for the tertiary screening, which was carried out essentially as described for the secondary screen.

After isolating single positive plaques from the human fetal brain library, 8 were chosen for further restriction enzyme and sequence analysis. The next step is to sequence these cDNA clones to determine whether any of them are homologous to the rat receptor, and if so, whether any of these candidates are full length clones, which is critical for functional expression studies of potential NPY/PYY receptor cDNAs.

## EXAMPLE II

### Sequencing cDNA Clones

#### 1. In vivo Excision of pBluescript Plasmid from Lambda ZAP Vector:

Phagemids were rescued from the lambda vector and transfected into XL1 Blue bacteria according to the Stratagene protocol. The single positive plaques from the agar plates were removed and placed into a solution containing SM buffer and chloroform in Eppendorf tubes. The samples were incubated at room temperature for one hour, with occasional vortexing, after which 0.2 ml of the plaque samples were added to 0.2 ml XL1-Blue cells (OD<sub>600</sub> =1.0) and 1 µl of R408 helper phage, and this was incubated at 37°C for 15 minutes. The 5 ml of 2X YT media (10 g NaCl, 10 g Yeast extract and 16 g Bacto-Tryptone/liter) were added to the samples and incubated for another 3 hrs at 37° C. Next, the samples were heated at 70° C for 20 minutes and centrifuged at 4000 x g for 5 min. The supernatant, containing the pBluescript

phagemid, was collected and 10  $\mu$ l was removed, and combined with 200  $\mu$ l XL1-Blue cells ( $OD_{600} = 1.0$ ), which was incubated at 37° C for 15 min. Subsequently, 20 and 50  $\mu$ l of these transfected cells were plated onto 100 mm LB/ampicillin plates and incubated overnight at 37° C.

## 2. Plasmid DNA Purification:

The Bluescript vector was purified from colonies using Promega Magic Miniprep system. The minipreps were performed according to the manufacturer's protocol. Overnight cultures were pelleted by centrifugation, and the pellets were resuspended in Cell Resuspension Solution (50 mM Tris-HCl, pH 7.5, 10 mM EDTA, 100  $\mu$ l/ml RNase A). Cell lysis solution (0.2 M NaOH, 1 mM EDTA) was added to the resuspended cells, and then the cells were neutralized in a solution of 2.55 M KOAc, pH 4.8. After spinning the samples in a microcentrifuge (14,000 x g for 5 min), the supernatant was collected and the DNA purification resin (Promega) was added before application to the mini-column. The mini-column containing the DNA-bound resin was rinsed with Column Wash Solution (100 mM NaCl, 10 mM Tris-HCl, pH 7.5, 2.5 mM EDTA and 50% ethanol); afterwards it was placed in a microcentrifuge tube, which was spun quickly to dry the resin. The plasmid DNA was eluted with pre-heated water (65-70°C) and respun, and the purified DNA was collected. After restriction enzyme characterization of the plasmid DNA, suitable clones were chosen for sequence analysis.

## 2.1 Manual Sequencing Procedure:

Prior to the sequencing reaction, the double-stranded Bluescript plasmid obtained from the miniprep procedure was alkali denatured (incubation at 37° C for 30 min. in 0.2 M NaOH, 0.2 mM EDTA), neutralized in 0.4 volume 5 M  $NH_4Ac$  and precipitated with 4 volume 100% ethanol at -70° C for 5 min., after which it was spun in a microcentrifuge and the pellet was washed with 70% ethanol. The sequencing was performed using The Sequenase Version 2.0 Sequencing kit (US Biochemical) and the sequencing reactions were carried out according to the manufacturer's instructions. Sterile water was added

- to the pelleted DNA, resuspended, and the following components were also added: 5X sequencing buffer (200 mM Tris-HCl, pH 7.5, 100 mM MgCl<sub>2</sub>, 250 mM NaCl), 3-4 pmol primer (T3, T7, SK, KS, M13 and M13 rev were synthesized on an ABI 394 DNA/RNA Synthesizer). The primers
- 5 were annealed to the plasmid by heating at 65°C for 2 min. and then cooled at room temperature. Each of the four termination mixtures were pipetted into microtiter plate wells. The 5X labeling Mix (7.5  $\mu$ M each of dGTP, dCTP, dTTP) was diluted with water, and the Sequenase was diluted in the enzyme dilution buffer (10 mM Tris-HCl, pH 7.5, 5 mM
- 10 DTT and 0.5 mg/ml BSA). For the labeling reaction, 0.1 M DTT, diluted labeling mix, 5  $\mu$ Ci [<sup>35</sup>S]-dATP (Amersham, >1000 Ci/mmol) and diluted sequenase were added to the annealing mixture and incubated at room temperature for 5 min. For the termination reaction, the labeling
- 15 reaction was added to each of the termination mixtures, and incubated for 5 min at 37° C. After this incubation, stop solution (95% formamide, 20 mM EDTA, 0.05% bromphenol blue and 0.05% xylene cyanol) was added to each reaction. The sequencing reactions were also carried out with the Pharmacia T7 sequencing kit using their sequencing protocol, which is similar to the procedure described above.
- 20 3. Automated Fluorescence Based Solid Phase Sequencing:
- Sequencing of the HY1-5 and HY1-7 clones were performed by using the manual dideoxy chain termination reaction using T7 DNA-polymerase and <sup>35</sup>S-ATP (described above) and by using a Taq-polymerase based dideoxy chain termination reaction with dye-labeled
- 25 2',3'-dideoxynucleoside triphosphates, where the sequencing reaction is separated on an automated DNA sequencing apparatus (Applied Biosystems 373A Sequencing System) that automatically collects sequence data and makes it possible to export sequences to a databank, where further analysis of the sequence can take place. The procedure
- 30 for sequencing the human Y1 receptor by using Taq-polymerase based dideoxy chain termination reactions with dye-labeled 2',3'-dideoxynucleoside triphosphates follows the protocol described below. All PCR reactions were performed on thermal cyclers from Perkin-Elmer.

3.1 Insert PCR of a DNA cloned in a plasmid and binding to a solid phase:

Two oligonucleotides (primers), JS1 (5'-GCGCGGATAACAATTTACACA-3') and JS2 (5'-

5 GCAGCACTGACCCTTTTGGGACCG-3') were constructed. They correspond to the sequences juxtaposed to the linker of the PUC plasmid and its derivatives, making it possible to do PCR amplification of a DNA cloned in the plasmid's linker. A second set of the JS-primers, called JS1B and JS2B, were modified by coupling biotin to the primer's 5'-end. Biotin is a protein that strongly binds a 66 kDa protein called Streptavidin. A PCR-reaction where one primer is biotinylated and the other is not generates a product that can be bound to a solid phase, in our case the Dynalbeads M-280 (ny Dynal, Norway) complexes between superparamagnetic polystyrene beads chemically bound to Streptavidin. Once the biotinylated product is bound to streptavidin the product can be denatured and the non-bound DNA can be washed away, resulting in single stranded DNA bound to the magnetic beads. The standard PCR insert amplification protocol is:

Ten picomoles of each primer (either JS1-JS2b or JS1B-JS2, depending on each strand that shall be sequenced), 10-100 picograms of plasmid-DNA in a PCR reaction that contains 50 mM KCl, 10 mM Tris-HCl (pH 8.4), 1.5 mM MgCl<sub>2</sub>, 0.8 mM dNTPs (Pharmacia), and 1 unit of Taq polymerase (Perkin-Elmer) in a 50 µl reaction volume.

Binding of the PCR generated product and separation of the two strands were achieved following the protocol:

1) Add 20 µl (0.2 mg) of the Dynalbeads to a magnetic Eppendorf stand. This will precipitate the beads immediately. Wash the Dynalbeads two times with a 200 µl SAMAG-solution (10 mM Tris-HCl, pH 7.0, 1 M NaCl, 0.1% Triton X-100).

2) Transfer the tubes with the beads to a non-magnetic stand. Add 20 µl of the PCR mixture with the biotinylated product and incubate on a shaker for 30 minutes at room temperature.

3) Transfer the tubes to a magnetic stand. Wash the beads once with 200 µl SAMAG.

4) Transfer the tubes with the beads to a non-magnetic stand. Denature the DNA by adding 200  $\mu$ l denaturation solution (0.1 M NaOH, 1 M NaCl). Incubate 15 minutes at room temperature.

5) Transfer the tubes to a magnetic stand. Wash once with 200  $\mu$ l of the denaturing solution. Wash once with 100  $\mu$ l of a 5 x PCR buffer (400 mM Tris-HCl pH 8.9, 100 mM Ammonium Sulfate, 25 mM  $MgCl_2$ ) diluted 1:5.

6) Transfer the tubes with the beads to a non-magnetic stand. Dissolve each sample in 6  $\mu$ l of ddH<sub>2</sub>O. Aliquot the dissolved beads into four tubes marked A (1  $\mu$ l), C (1  $\mu$ l), G (2  $\mu$ l), and T (2  $\mu$ l). (See below "B. Aliquoting the reagents")

### 3.2 Fluorescent DNA Taq sequencing:

The dye primers, M13, M13rev, T3 and T7 and Taq-sequencing kit were purchased from ABI. Sequencing reactions were performed according to the manufacturer's protocol.

#### A. Diluting the enzyme

Mix 0.5  $\mu$ l AmpliTaq DNA polymerase (8 units/ $\mu$ l) with a 1.0  $\mu$ l 5 x PCR buffer (400 mM Tris-HCl pH 8.9, 100 mM Ammonium Sulfate, 25 mM  $MgCl_2$ ) and 5.5  $\mu$ l H<sub>2</sub>O.

#### B. Aliquoting the reagents

Aliquot the reagents into four 0.5 ml microcentrifuge tubes according to the following protocol:

Reagent	A	C	G	T
d/ddNTP Mix	1 $\mu$ l	1 $\mu$ l	2 $\mu$ l	2 $\mu$ l
Dye primer (0.4 pmol/ $\mu$ l)	1 $\mu$ l	1 $\mu$ l	2 $\mu$ l	2 $\mu$ l
5 x PCR buffer	1 $\mu$ l	1 $\mu$ l	2 $\mu$ l	2 $\mu$ l
DNA template	1 $\mu$ l	1 $\mu$ l	2 $\mu$ l	2 $\mu$ l
Diluted Taq	1 $\mu$ l	1 $\mu$ l	2 $\mu$ l	2 $\mu$ l
Total Volume	5 $\mu$ l	5 $\mu$ l	10 $\mu$ l	10 $\mu$ l

Overlay each of the four reactions with about 20  $\mu$ l mineral oil.

#### C. Cycling the reactions

Place the tubes in a thermal cycler preheated to 95°C. For the present invention, a modified PCR cycle was used which included 1

minute at 94°C, 1 min at 55° C, and 1 min at 72° C for 25 cycles followed by a soak file at 4° C. These PCR conditions appeared to be as efficient as the conditions recommended by the manufacturer.

D. Concentrating the sample

5 In a separate tube mix 80 µl 95% ethanol with 1.5 µl 3 M sodium acetate (pH 5.3). Pipette the extension reaction from the bottom of each of the four tubes into the ethanol mixture. Mix thoroughly. Precipitate at room temperature for 10-15 minutes. Spin in a microcentrifuge for 30 minutes. Rinse the pellet with 70% ethanol and spin for another 5 minutes. Dry the pellet in a vacuum centrifuge for 1-3 minutes.

E. Loading the sample

Prior to loading, samples were resuspended in 6 µl of deionized formamide/50 mM EDTA (pH 8.0) in the proportions 5:1. Heat the sample at 90° C for 2 min. and load immediately on a pre-electrophoresed gel.

3.3 The ABI 373A Apparatus

The apparatus is based on a four-dye, one lane, scanned laser technology. Conventional 6% polyacrylamide gels are used ((57 g acrylamide, 3 g bis-acrylamide, 450 g urea and 100 ml 10 x TEB)/liter).

20 The settings on the machine to perform a 14 hour long run are based on the manufacturer's recommendations: 2500 V, 40 mAMP, 30 W, 40°C.

The chromatograms obtained after a gel run on the ABI 373A (equivalent to the autoradiographs when reactive isotopes are used) were examined in detail by using Seqed™, the Macintosh compatible software from ABI, that allows editing of the collected sequence. The sequences were then exported to a VAX computer that has access to the UWGCG (University of Wisconsin Genetics Computer Group) package and many sequence banks (e.g. GenBank, EMBL, Swiss-prot.). The sequences were transferred by using the shareware Xferit 1.5 by Falkenburg.

30 The alignment of all the sequences obtained from manual and automated sequencing was created as a project called HCY1 by using a program package consisting of e.g. Gelstart, Gelenter, and Geloverlap. Gelassemble All computer work was done by using a Macintosh LC connected to a Localtalk net, allowing communication with the VAX



computer via the Public software Telnet MacTCP communication program (NSCA Software Development).

#### 4. Gel Electrophoresis:

5 The sequencing reactions were incubated at 75° C for 2 min before they were run on 6% acrylamide gels (57 g acrylamide, 3 g bis-acrylamide, 480 g urea/liter in 1X TBE buffer). The gels were 0.4 mm thick and 30 cm x 38 cm in size. After the samples were run, the gel was dried (1 hr at 80° C) with a gel dryer, and exposed to XAR-5 film for 18-72 hrs.

10 Enzyme digestion of rescued plasmids revealed several overlapping sibling clones, of which suitable clones, i.e. the longest (hY1-5) and those containing overlapping coding regions, were selected for sequencing analysis. In addition, 4 specific synthetic primers (3 forward primers: CTCTTGCTTATGGA/GGCTGTGA,  
15 TATGTAGGTATTGCTGTGATTTG, TATACCACTCTTCTC/TT/CTGGTGCTG and one reverse primer, CTGGAAGTTTTTGTTCAGGAAT/CCCA were used for manual sequencing of the hY1-5 clones and its deletion constructs (Eco RV, Xho, Nsi-Sac and Nsi-Eco RV). The clone of interest (hY1-5) was then further characterized by Northern blot hybridization in order to  
20 estimate its mRNA size, and by Northern blots and RT-PCR to examine its distribution. Southern hybridizations were also performed to determine the number of copies of this gene present in the human genome.

25

### EXAMPLE III

#### Northern and Southern Hybridization

##### 1. Preparation of mRNA:

30 The mRNA from several neuroblastoma cell lines (SK-N-MC, IMR, SH-SY-5Y, LAN1, LAN2, LAN5, 1523, 2674) were purified by standard guanidinium isothiocyanate/oligo (dT)-cellulose methods. Briefly, cultured cells were homogenized in guanidinium thiocyanate homogenization buffer (4 M guanidinium thiocyanate, 0.1 M Tris-HCl, pH 7.5, 1% beta-mercaptoethanol, 0.5% sodium lauryl sarcosinate) and the resulting lysate was centrifuged (5000 x g, 20 min). After collecting

the supernatant, 0.1 vol 3M sodium acetate (pH 5.2) and 0.5 vol cold 100% ethanol were added and incubated on ice for 2 hrs. The nucleic acid was pelleted by centrifugation (as above), and the pellet was resuspended in a second guanidinium thiocyanate buffer (4 M guanidinium thiocyanate, 0.1 M sodium acetate, pH 7.0, 1 mM DTT, 20 mM EDTA, pH 8.0). The nucleic acid was precipitated in 0.5 vol cold 100% ethanol and incubated at -20° C for 2 hrs. The nucleic acid was pelleted as before, and precipitated twice more. The final pellet was resuspended in 20 mM EDTA (pH 8.0) and 1 volume chloroform: 1-butanol (4:1). This was recentrifuged as before and extraction with phenol/chloroform/isoamyl alcohol was repeated. To precipitate the RNA, 3 volumes of 4 M sodium acetate (pH 7.0) was added to the last aqueous phase, incubated at -20° C for 2 hrs before centrifugation, after which two more rounds of ethanol precipitation were carried out and the RNA was dissolved in water. The total RNA was heated at 65° C for 5 min before addition of loading buffer (20 mM Tris-HCl, pH 7.6, 0.5 M NaCl, 1 mM EDTA, pH 8.0, 0.1% sodium lauryl sarcosinate), which was then applied onto oligo (dT)-cellulose columns. The columns were washed with the loading buffer and the poly (A) RNA was eluted with the elution buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA, pH 8.0, 0.05 SDS) and the collected poly(A) was precipitated in 0.1 volume 3 mM sodium acetate (pH 5.2) and 2.5 volumes cold ethanol. After incubation at -20°C, this RNA was pelleted by centrifugation (10,000 x g for 30 min) and was dissolved in water.

## 2.5 2. Transfer of mRNA to Nylon Membranes:

The mRNA (few µg) was run on formaldehyde-containing agarose gels and transferred to nylon filters by capillary elution and RNA was crosslinked to the nylon membranes using a UV Stratalinker 1800 (Stratagene).

## 3.0 3. Hybridization Conditions:

The probe, a 1.4 kb XhoI-EcoRI fragment of hY1-5, was labeled with <sup>32</sup>P as described earlier in Example I. The prehybridization conditions were carried out as described for cDNA screening, except that 2X SSC was used instead of 1 M NaCl. The filters were washed

using the previously outlined conditions (for cDNA screening), with the exception that the final two washes were done at 65° C in 0.2 X SSC and 0.1% SDS. The filters were exposed to film as described earlier.

#### 4. Southern Hybridization:

5       The human leucocyte genomic DNA was prepared (by standard procedure) and digested with restriction enzymes. This DNA was then run on a 1% agarose gel and the DNA was transferred to filters as described before. The probe, hybridization and washes were also as described for the Northern hybridization.

10       The Northern hybridization showed that the hY1-5 fragment hybridized to a single 3.5 kb transcript in SK-N-MC, which was known to express Y1 receptors, whereas the probe failed to identify Y1 transcripts in several other neuroblastoma cell lines. The Southern hybridization results suggests that the genome contains a single Y1  
15       receptor gene. In order to confirm that the cDNA clones were the human Y1 receptor, specific primers synthesized for sequencing the clones, were used in RT-PCR.

---

### EXAMPLE IV

#### 20                   Reverse Transcription-PCR

##### 1. RNA Preparation:

25       A. The mRNA was prepared from SK-N-MC cells using the Fast Track mRNA Isolation Kit (Invitrogen), following their instructions. In brief, cells were washed in PBS, pelleted by centrifugation, resuspended and lysed in lysis buffer (kit), and subsequently  
30       homogenized in a Dounce homogenizer. The lysate was passed through a 21 gauge needle several times, incubated at 45° C for 2 hrs, and added to pre-equilibrated oligo (dT) cellulose and incubated for another hour at room temperature with shaking. The oligo (dT) cellulose-bound  
mRNA was pelleted by centrifugation and resuspended in binding buffer (kit); this was repeated three times before the samples were loaded onto spin columns and quickly spun to remove excess buffer. Next, the oligo (dT) cellulose was resuspended in elution buffer and respun, after which the eluted mRNA was collected and precipitated in 0.15 volume 2

M sodium acetate and 2.5 volumes 100% ethanol. The RNA was pelleted and resuspended in elution buffer, and stored at -70° C until used.

B. Cytoplasmic RNA was prepared from human cultured circumflex coronary artery smooth muscle cells. As before, cells were  
5 washed in PBS, centrifuged, and resuspended in lysis buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 5 mM MgCl<sub>2</sub>, 0.5% NP-40; also added RNase inhibitor). After incubating on ice for 5 min, the lysate was centrifuged, and the supernatant was collected. Proteinase K (100 µg in 100 mM Tris, pH 7.5, 12.5 mM EDTA, 0.15 M NaCl, 1% SDS) was added  
10 to the supernatant, and incubated for 15 min at 37° C. The RNA was extracted with 2 sets of phenol/chloroform/isoamyl alcohol extractions and precipitated with 1 volume isopropanol. The cytoplasmic RNA was pelleted by centrifugation and redissolved in water.

15 2. Reverse Transcription Reaction:

The reverse transcription reaction was performed using the cDNA Cycle kit (Invitrogen). Approximately 1 µg of SK-N-MC mRNA (or 5 µg of circumflex coronary artery smooth muscle cell total RNA) was used in each reaction, which consisted of 10 mM MeHgOH, 0.1 M beta-  
20 mercaptoethanol, 0.2 µg of oligo dT primer, RNase inhibitor, 5X buffer, 1 mM dNTPs and 5 units reverse transcriptase. For the total RNA sample, the primer was first incubated at 65° C for 2 min before the addition of the other reagents. The samples were incubated at 42° C for one hour, followed by another incubation at 95° C for 3 min, after which  
25 another 5 units of reverse transcriptase was added and the cDNA synthesis was repeated. The resulting cDNA was used directly for PCR analysis.

3. Polymerase Chain Reaction:

The four primers synthesized for the sequencing reaction  
30 (Example II) were also used for the PCR reaction. The same PCR reagents were used as described in the probe preparation section (Example I; 50 pmol of forward and reverse primers), however, the cycling conditions were slightly different: 5 min at 95° C for 1 cycle, then 1 min at 93° C, 2 min at 55° C and 2 min at 72° C for 35 cycles.

The resulting PCR products were run on 1.5% agarose gels along with molecular weight markers to estimate their sizes.

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#### EXAMPLE V

5

#### Functional Expression

The cDNA of the human NPY Y1 receptor was transfected into COS 1 cells. This was done in order to establish the functional identity of our clone. Following the transfection procedure described below, the cells were studied with respect to: (i) radioreceptor binding using  
10 <sup>125</sup>I-peptide YY (PYY); (ii) cyclic AMP accumulation by use of radioimmunoassay kit (Advanced Magnetics); and (iii) <sup>45</sup>Ca<sup>2+</sup> influx into the cells from the extracellular space. The two latter so-called second messenger responses were elicited by stimulation of the cells with NPY. In all three types of assay, cells not transfected with the Y1  
15 receptor cDNA were used as controls to verify that these COS 1 cells do not normally possess Y1 receptors.

All steps of the transfection procedure were carried out under a sterile hood with the exception of purification and ethanol precipitation of the plasmid. Dulbecco's Modified Eagle Media (DMEM)  
20 contains 1% Penicillin-Streptomycin in all procedures unless otherwise specified.

1. COES 1 cells, passages 6 to 17, were maintained in T75 flask in DMEM supplemented with 10% fetal calf serum (GIBCO-BRL) at 37° C and 95% humidity under 5% CO<sub>2</sub> avoiding confluency until used.

25 2. The day before transfections were performed, cells were trypsinized and washed with 25 ml of DMEM containing 10% NuSerum (Collaborative Research; Catalogue #5000) to completely remove trypsin. After trituration, cells were subcultured to the density of 220,000 cells per 35 mm plate in 2 ml DMEM with 10% NuSerum. Allow approximately 20  
30 hours for cells to attach to plates under the same culture-incubator condition described above.

3. On the day of transfection, the purified plasmid (cDNA of human NPY Y1 receptor in PCDM8 vector (Invitrogen) was precipitated with ethanol and dissolved in sterile 20 mM HEPES buffer (pH 7.4) containing 150 mM

- NaCl to the final concentration of 0.1 mg/ml. Mix 2  $\mu$ g plasmid with 15  $\mu$ g DEAE-dextran (stock solution 50 mg/ml) in a volume less than 50  $\mu$ l in a polystyrene tube. The final concentration of DEAE-dextran should be 500  $\mu$ g/ml after mixed with media for transfections. Leave the
- 5 mixture at room temperature until the media for the transfection is prepared (10 minutes). At this concentration of plasmid, normally no precipitation was formed, however, if any precipitate was visible, the volume of buffer was increased to 100  $\mu$ l.
4. Combine 1.5 ml DMEM containing 10% NuSerum with chloroquin
- 10 phosphate (stock solution 75 mM) to the final concentration of 75  $\mu$ M, then add the media to the plasmid DEAE-dextran mixture, mix and lay it over cells. Typically the transfection mixtures were prepared in a batch when it was performed in a number of 35 mm plates. For example, for 50 plates of 35 mm diameter, 100  $\mu$ g plasmid was mixed
- 15 with 750  $\mu$ l of 50 mg/ml DEAE-dextran, to which 75 ml DMEM media with 10% NuSerum and 75  $\mu$ M chloroquin phosphate was added. After thorough mixing by pipetting up and down, 1.5 ml of the mixture was added to each plate.
5. After incubation at 37° C and 95% humidity under 5% CO<sub>2</sub> for 3.5
- 20 hours, not exceeding 4 hours, cells were shocked by incubating in 2 ml 10% DMSO (in Hank's balanced salt solution with Mg<sup>2+</sup> and Ca<sup>2+</sup>) for 1 minute. Cells were then washed with 3 ml DMEM containing 10% NuSerum and further incubated for 64-72 hours in 7 ml DMEM with 10% fetal calf serum under the incubator condition described above.
- 25 6. For the transfection using 145 mm plates, cells were subcultured to  $2.8 \times 10^6$ /plate in 15 ml DMEM with 10% NuSerum. Immediately before transfection, the media was replaced with 9 ml of fresh one containing 75  $\mu$ M chloroquin phosphate. Transfection mixture for each plate contained 25  $\mu$ g plasmid and 100  $\mu$ l 50 mg DEAE-dextran in 1 ml DMEM
- 30 with 10% NuSerum and 75  $\mu$ M chloroquin phosphate. The mixture was then added to the cells by dropwise over the media and the plate was gently swirled to achieve the uniform mixing.
7. The cells grown in 145 mm plates were washed three times, harvested and suspended in 50 mM ice-cold Tris-HCl buffer (pH 7.4)

with 5 mM EDTA and 1 mM  $\beta$ -mercaptoethanol and then homogenized using Polytron (Brinkman; setting 6) for 10 sec. The homogenate was centrifuged at 1,000 x g for 10 min using a swinging bucket rotor. The supernatant was then subjected to ultracentrifugation at 100,000 x g for 30 min. The resulting pellet was resuspended by Polytron homogenizer in fresh binding buffer (137 mM NaCl, 5.4 mM KCL, 0.44 mM  $\text{KH}_2\text{PO}_4$ , 1.26 mM  $\text{CaCl}_2$ , 0.81 mM  $\text{MgSO}_4$ , 20 mM HEPES, 0.3% bovine serum albumin and 0.01 % bacitracin; pH 7.4) and membranes from  $2 \times 10^6$  cells were used per assay tube in a final volume of 0.4 ml. Samples were then incubated with  $^{125}\text{I}$ -peptide YY ( $^{125}\text{I}$ -PYY (New England Nuclear); 2200 Ci/mmol; 22°C; 100 min)). In displacement-type experiments, 0.1 nM radioligand was used. The incubations were terminated by centrifugation (Eppendorf Microfuge) for 2 min, followed by washing of the pellets' surface by 1 ml ice-cold buffer. Pellets were counted in a Packard gamma-counter. Binding data were analyzed using LIGAND<sup>TM</sup> and KINETIC<sup>TM</sup> (Biosoft).

8. Cells grown in 35 mm dishes were used for  $^{45}\text{Ca}^{2+}$  influx studies or cAmp studies:

A.  $^{45}\text{Ca}^{2+}$  influx:

Prior to influx studies, transfected (60 hours earlier) and control transfected COS 1 cells were washed three times with 1.5 ml of the above described binding buffer solution fortified with 10 mM glucose.  $^{45}\text{Ca}^{2+}$  influx (Amersham Corp; 3-5  $\mu\text{M}$ ; 6-10  $\mu\text{Ci}$ ) was studied over 2 minutes with or without agonist. Final volumes were 1 ml and experiments were performed at room temperature with solutions kept at 37° C prior to use. The uptake was terminated by rapid aspiration of the incubation mixture. Cells were then washed four times with ice cold buffer in order to remove the residual extracellular radioactivity and lysed with 1 ml of lysis buffer (8 M urea, 3 M acetic acid, and 2% Nonidet P-40). The amount of  $^{45}\text{Ca}^{2+}$  influx into the cells was estimated by counting the disintegrations per minute (DPM) of lysate aliquots in a liquid scintillation system after mixing with Formula 989 (New England Nuclear).

B. Cyclic AMP accumulation determination:

Sixty hours after transfection (35 mm wells) cells were equilibrated in the HEPES-based buffer described above for one hour. Phosphodiesterase inhibitor, methylisobutylxanthine (500  $\mu$ M) was present throughout the cAMP experiments. Ten minutes after addition of 100 nM NPY, the cells were challenged with 5  $\mu$ M forskolin. Fifteen minutes later, the reaction was terminated by adding 1 ml of ethanol to the 1 ml of incubation mixture. The cells were harvested from each well into individual tubes, the wells were washed with another 1 ml of ethanol, and the washings were combined. The cells were then sonicated and left on ice for 10 minutes. Precipitated proteins were separated by centrifugation, the precipitates were washed once with 1 ml of ethanol, and the supernatants were combined. The final ethanol extract was evaporated under vacuum and the residue dissolved in assay buffer supplied with the cyclic AMP radioimmunoassay kit. Assay for cyclic AMP was carried out using the non-acetylated protocol, precisely as described by the kit manufacturer.

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As described above, thirteen human fetal brain cDNA clones hybridizing under low stringency conditions to a human PCR fragment corresponding to the rat "orphan" receptor, FC5, were isolated from 200,000 screened clones. Eight of these were rescued from the phage vector and were found to share several restriction sites, yet displaying distinct insert sizes. The independent clones hY1-5 and hY1-7 were used for DNA sequencing. Clone hY1-5 had an insert of 2.1 kb that included the entire coding region of the putative homolog of FC5. We found that clone hY1-5 contained 200 bp of 5' untranslated sequence. Preceding the ATG initiation codon at position 197, all three reading frames were interrupted by termination codons. No polyadenylation signal was found at the 3' end of clone hY1-5.

The Nucleotide sequence of the human Y1-receptor cDNA is depicted below:

```

CCTTCTTTTAA TGAAGCAGGA GCGAAAAAGA CAAATTCCAA AGAGGATTGT   50
TCAGTTCAAG GGAATGAAGA ATTCAGAATA ATTTTGGTAA ATGGATTCCA   100
ATATGGGGAA TAAGAATAAG CTGAACAGTT GACCTGCTTT GAAGAAACAT   150

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	ACTGTCATT TGICTAAAAT AATCTATAAC AACCAAACCA ATCAAA	196
	ATG AAT TCA ACA TTA TTT TCC CAG GTT GAA AAT CAT TCA	235
	GTC CAC TCT AAT TTC TCA GAG AAG AAT GCC CAG CTT CTG	274
	GCT TTT GAA AAT GAT GAT TGT CAT CTG CCC TTG GCC ATG	313
5	ATA TTT ACC TTA GCT CTT GCT TAT GGA GCT GTG ATC ATT	352
	CTT GTC TCT GGA AAC CTG GCC TTG ATC ATA ATC ATC TTG	391
	AAA CAA AAG GAG ATG AGA AAT GTT ACC AAC ATC CTG ATT	430
	GTG AAC CTT TCC TTC TCA GAC TTG CTT GTT GCC ATC ATG	469
	TGT CTC CCC TTT ACA TTT GTC TAC ACA TTA ATG GAC CAC	508
10	TGG GTC TTT GGT GAG GCG ATG TGT AAG TTG AAT CCT TTT	547
	GTG CAA TGT GTT TCA ATC ACT GTG TCC AAT TTC TCT CTG	586
	GTT CTC AAT GCT GTG GAA CGA CAT CAG CTG ATA ATC AAC	625
	CCT CGA GGG TGG AGA CCA AAT AAT AGA CAT GCT TAT GTA	664
	GGT AAT GCT GTG AAT TGG GTC CTT GCT GTG GCT TCT TCT	703
15	TTG CCT TTC CTG ATC TAC CAA GTA ATG ACT GAT GAG CCG	742
	TTC CAA AAT GTA ACA CTT GAT GCG TAC AAA GAC AAA TAC	781
	GTG TGC TTT GAT CAA TTT CCA TCG GAC TCT CAT AGG TTG	820
	TCT TAT ACC ACT CTC CTC TTG GTG CTG CAG TAT TTT GGT	859
	CCA CTT TGT TTT ATA TTT AAT TGC TAC TTC AAG ATA TAT	898
20	ATA CGC CTA AAA AGG AGA AAC AAC ATG ATG GAC AAG ATG	937
	AGA GAC AAT AAG TAC AGG TCC AGT GAA ACC AAA AGA ATC	976
	AAT ATC ATG CTG CTC TCC AAT GTG GTA GCA TTT GCA GTC	1015
	TGC TGG CTC CCT CTT ACC ATC TTT AAC ACT GTG TTT GAT	1054
	TGG AAT CAT CAG ATC ATT GCT ACC TGC AAC CAC AAT CTG	1093
25	TTA TTC CTG CTC TGC CAC CTC ACA GCA ATG ATA TCC ACT	1132
	TGT GTC AAC CCC ATA TTT TAT GGG TTC CTG AAC AAA AAC	1171
	TTC CAG AGA GAC TTG CAG TTC TTC TTC AAC TTT TGT GAT	1210
	TTC CGG TCT CGG GAT GAT GAT TAT GAA ACA ATA GCC ATG	1249
	TCC ACG ATG CAC ACA GAT GTT TCC AAA ACT TCT TTG AAG	1288
30	CAA GCA AGC CCA GTC GCA TTT AAA AAA ATC AAC AAC AAT	1327
	GAT GAT AAT GAA AAA ATC TGA AAC TAC TTA TAG CCT ATG	1366
	GTC CCG GAT GAC ATC TGT TTA AAA ACA AGC ACA ACC TGC	1405
	AAC ATA CTT TGA TTA CCT GTT CTC CCA AGG AAT GGG GTT	1444
	GAA ATC AAT TGA AAA TGA CTA AGA TTT TCT TGT CTT GCT	1483

TTT TAC AGT TTT GAC CAG ACA TCT TTG AAG TGC TTT TTG 1522  
TGA ATT TAC CAG 1534

Within this sequence, the structural gene for the Y-1 receptor consists of the sequence between nucleotide 197 and 1534. The deduced amino acid sequence of the human Y1-receptor taken from this cDNA sequence is:

	Met	Asn	Ser	Thr	Leu	Phe	Ser	Gln	Val	Glu	Asn	His	Ser	Val	His	
					5					10					15	
10	Ser	Asn	Phe	Ser	Glu	Lys	Asn	Ala	Gln	Leu	Leu	Ala	Phe	Glu	Asn	
					20					25					30	
	Asp	Asp	Cys	His	Leu	Pro	Leu	Ala	Met	Ile	Phe	Thr	Leu	Ala	Leu	
					35					40					45	
	Ala	Tyr	Gly	Ala	Val	Ile	Ile	Leu	Gly	Val	Ser	Gly	Asn	Leu	Ala	
					50					55					60	
15	Leu	Ile	Ile	Ile	Ile	Leu	Lys	Gln	Lys	Glu	Met	Arg	Asn	Val	Thr	
					65					70					75	
	Asn	Ile	Leu	Ile	Val	Asn	Leu	Ser	Phe	Ser	Asp	Leu	Leu	Val	Ala	
					80					85					90	
20	Ile	Met	Cys	Leu	Pro	Phe	Thr	Phe	Val	Tyr	Thr	Leu	Met	Asp	His	
					95					100					105	
	Trp	Val	Phe	Gly	Glu	Ala	Met	Cys	Lys	Leu	Asn	Pro	Phe	Val	Gln	
					110					115					120	
	Cys	Val	Ser	Ile	Thr	Val	Ser	Ile	Phe	Ser	Leu	Val	Leu	Ile	Ala	
					125					130					135	
25	Val	Glu	Arg	His	Gln	Leu	Ile	Ile	Asn	Pro	Arg	Gly	Trp	Arg	Pro	
					140					145					150	
	Asn	Asn	Arg	His	Ala	Tyr	Val	Gly	Ile	Ala	Val	Ile	Trp	Val	Leu	
					155					160					165	
30	Ala	Val	Ala	Ser	Ser	Leu	Pro	Phe	Leu	Ile	Tyr	Gln	Val	Met	Thr	
					170					175					180	
	Asp	Glu	Pro	Phe	Gln	Asn	Val	Thr	Leu	Asp	Ala	Tyr	Lys	Asp	Lys	
					185					190					195	
	Tyr	Val	Cys	Phe	Asp	Gln	Phe	Pro	Ser	Asp	Ser	His	Arg	Leu	Ser	
					200					205					210	
35	Tyr	Thr	Thr	Leu	Leu	Leu	Val	Leu	Gln	Tyr	Phe	Gly	Pro	Leu	Cys	
					215					220					225	
	Phe	Ile	Phe	Ile	Cys	Tyr	Phe	Lys	Ile	Tyr	Ile	Arg	Leu	Lys	Arg	
					230					235					240	
40	Arg	Asn	Asn	Met	Met	Asp	Lys	Met	Arg	Asp	Asn	Lys	Tyr	Arg	Ser	
					245					250					255	
	Ser	Glu	Thr	Lys	Arg	Ile	Asn	Ile	Met	Leu	Leu	Ser	Ile	Val	Val	
					260					265					270	

	Ala	Phe	Ala	Val	Cys	Trp	Leu	Pro	Leu	Thr	Ile	Phe	Asn	Thr	Val
					275					280					285
	Phe	Asp	Trp	Asn	His	Gln	Ile	Ile	Ala	Thr	Cys	Asn	His	Asn	Leu
					290					295					300
5	Leu	Phe	Leu	Leu	Cys	His	Leu	Thr	Ala	Met	Ile	Ser	Thr	Cys	Val
					305					310					315
	Asn	Pro	Ile	Phe	Tyr	Gly	Phe	Leu	Asn	Lys	Asn	Phe	Gln	Arg	Asp
					320					325					330
10	Leu	Gln	Phe	Phe	Phe	Asn	Phe	Cys	Asp	Phe	Arg	Ser	Arg	Asp	Asp
					335					340					345
	Asp	Tyr	Glu	Thr	Ile	Ala	Met	Ser	Thr	Met	His	Thr	Asp	Val	Ser
					350					355					360
	Lys	Thr	Ser	Leu	Lys	Gln	Ala	Ser	Pro	Val	Ala	Phe	Lys	Lys	Ile
					365					370					375
15	Asn	Asn	Asn	Asp	Asp	Asn	Glu	Lys	Ile						
					380										

The predicted Y1-receptor sequence shows 93% identity to that deduced from the rat FC5 clone, which is proposed to correspond to a rat Y1-receptor. Of the 24 amino acid replacements, seven occur in the N-terminal extracellular part and nine occur in transmembrane region (TM) 4 and the following extracellular loop. The human Y1-sequence has two additional amino acids as compared to its rat counterpart, one in the N-terminal extension and one near the C-terminus. It is notable that the sequence DRY (Asp-Arg-Tyr), which follows TM3 in most receptors belonging to the G-protein-coupled receptor superfamily, reads ERH (Glu-Arg-His) in the Y1 sequences of both human and rat. Most other positions which are highly conserved in the receptor superfamily are also conserved in the predicted Y1-sequences.

The peptides according to the present invention can be synthesized by any suitable method, such as by exclusively solid-phase techniques, by partial solid-phase techniques, by fragment condensation or by classical solution addition. Synthetic Y1-receptor according to the present invention may also be entirely or partially synthesized by recently developed recombinant DNA techniques, which may likely be used for large-scale production.

For example, the techniques of exclusively solid phase synthesis are set forth in "Solid Phase Peptide Synthesis" by Stewart and Young, Freeman & Company, San Francisco (1969), and exemplified in US Patent

4,105,603; fragment condensation methods of synthesis are exemplified in US Patent 3,972,859; and other available synthesis protocols are exemplified in US Patents 3,842,067 and 3,862,925.

5 Synthesis by use of recombinant DNA techniques, for purposes of the present invention, should be understood to include the suitable employment of a structural gene coding for all or an appropriate section of the Y1-receptor to transform a microorganism, using an expression vector including an appropriate promoter and operator together with the structural gene, and causing the transformed microorganism to express  
10 the peptide or such a synthetic peptide fragment. For example, either the complete cDNA sequence for the Y1-receptor peptide depicted above or the structural gene sequence from nucleotide 197 to nucleotide 1534 may be used in recombinant techniques. A non-human animal may also be used to produce the peptide by gene-farming using such a structural  
15 gene or cDNA in the microinjection of embryos.

Such recombinant techniques are well known in the field of biotechnology, and can be easily used given the description presented herein.

20 When the peptides are not prepared using recombinant DNA technology, they are preferably prepared using solid phase synthesis, such as that described by Merrifield [see J. Am. Chem. Soc. 85:2149 (1964)], although other equivalent chemical syntheses known in the art can also be used as previously described.

25 The presence of Y1-receptor mRNA in various human cultured cells was investigated by (1) Northern hybridizations using human Y1-probe (Fig. 1) and (2) by RT-PCR using specific human Y1-primers (data not shown). Both methods showed the human neuroblastoma cell line, SK-N-MC to express Y1-receptors (Fig. 1); this particular cell line has been viewed as a model system for studies on Y1-receptors. The size of  
30 the single hybridizing transcript in SK-N-MC is approx. 3.5 kb. By Northern hybridization, we failed to identify Y1-transcripts in several other neuroblastoma cell lines, i.e. IMR (Fig. 1), SH-SY-5Y, LAN1, LAN2, LAN5, 1523 or 2674 (not shown). By using one reverse and three forward primers in RT-PCR, we confirmed the presence of the Y1-

receptor in SK-N-MC, and, in addition, PCR products of the same sizes (350, 520 and 850 bp) were also detected in human cultured circumflex coronary artery smooth muscle cells. The latter observation is in agreement with previous suggestions that the Y1-receptor is expressed in vasculature. The same RT-PCR protocol, which again yielded 350, 520 and 850 bp products when the human fetal brain library was used as template, failed to yield any detectable product in the neuroblastoma cell line, SK-N-BE(2), which is thought to express Y2-receptors. Southern hybridization to human genomic DNA followed by high-stringency washes (Fig.1) suggest that the genome contains a single Y1-receptor gene.

The insert of hY1-5 was transferred to the mammalian expression vector, pCDM8, and used to transfect COS1 cells. Such transfected cells were used for studies on (1) radioligand, i.e.  $^{125}\text{I}$ -PYY, binding and (2) second messenger, i.e.  $\text{Ca}^{2+}$  and cAMP, analyses. As a negative control for all these assays, identical COS1 cells transfected with the rat type-1 angiotensin receptor in the same pCDM8 expression vector were used; in such control cells little or no specific  $^{125}\text{I}$ -PYY binding was observed, and no second messenger responses to NPY or PYY.

Radioligand binding assays in membranes prepared from the hY1-5 transfected cells indicate that the clone encodes a protein with the pharmacological characteristics typical of a Y1-receptor type. The dissociation constant ( $K_d$ ) was  $0.86 \pm 0.09$  nM ( $n = 4$ ; mean  $\pm$  SEM), assuming a single-site fit and equal affinity of (porcine)  $^{125}\text{I}$ -PYY and unlabeled (porcine) PYY. This  $K_d$  is similar to that observed in SK-N-MC and other cell types. The pharmacological profile of ligands competing for  $^{125}\text{I}$ -PYY binding to the expressed clone, illustrated in Fig. 2, is also consistent with that of a Y1-receptor. The potency series of  $\text{PYY} \geq \text{NPY} \geq [\text{Leu}^{31}, \text{Pro}^{34}]\text{NPY} \gg \text{NPY2-36} > \text{C2-NPY} > (\text{human}) \text{PP} > \text{NPY13-36} > \text{NPY18-36}$  was determined (Fig.2); similar rank orders of potency have been observed in various vascular smooth muscle cells [see Br. J. Pharmacol. 105:45 (1992)] and SK-N-MC [see Life Sci. 50 PL7-PI12 (1992)]. Human NPY was equipotent with porcine NPY (not shown).

Two second messenger responses frequently associated with Y1-receptors are influx of  $\text{Ca}^{2+}$ , which is not necessarily associated with activation of phosphoinositidase C, and inhibition of cAMP accumulation. Thus, 100 nM of NPY and PYY was found to accelerate in flux of  $^{45}\text{Ca}^{2+}$ , as studied over 2 min, by  $135 \pm 17\%$  and  $157 \pm 23\%$  of control, respectively (mean  $\pm$  SEM;  $n = 6$ ; two different experiments;  $p < 0.001$ ) in hY1-5 transfected COS1 cells; this is similar to the case for endogenous Y1-receptors in SK-N-MC. Control transfected cells did not respond to either NPY or PYY (100 nM). Another well-established characteristic of Y1-receptors, e.g. in SK-N-MC, is the coupling to reduced cAMP accumulation. Likewise, stimulation of the *de novo* expressed Y1-receptor by 100 nM NPY inhibited forskolin (5  $\mu\text{M}$ ) elevated accumulation of cAMP in the COS1 cells by  $47 \pm 55\%$  (mean  $\pm$  SEM;  $n = 6$ ; similar results obtained in two different experiments;  $p < 0.01$ ). In the latter experiments, in which the phosphodiesterase inhibitor, methylisobutylxanthine (500  $\mu\text{M}$ ) was present throughout, 100 nM NPY also reduced basal cAMP concentrations from  $240 \pm 14$  to  $123 \pm 4\%$  (pmol of cAMP per 35 mm well; means  $\pm$  SEM;  $n = 6$ ;  $p < 0.001$ ).

The heterologously expressed Y1-receptor described herein is thus similar to the endogenous Y1-receptor in brain, and neuroblastoma [5,7] and vascular smooth muscle cells with respect to binding and second messenger properties [see NY Acad. Sci. 611:7 (1990)]. Sequence analysis strongly indicated that the Y1-receptor belongs to the G-protein-coupled receptor superfamily. The human Y1-sequence is, however, only distantly related to the two proposed NPY receptors that have appeared in the literature very recently [see Mol. Pharmacol. 40:869 (1991), and J. Biol. Chem. 267:9 (1992)]. The portion of the sequence spanning the TM regions of hY1-5 shows only 21% and 23% identity with proposed bovine and Drosophila NPY receptors, respectively; the Y1-sequence appears more closely related to tachykinin receptors (29% sequence identity) [see Ann. NY Acad Sci. 632:53 (1991)], and it is similar to the human somatostatin type 1 receptor (23% identity) [see Proc. Natl. Acad. Sci. USA 89:251 (1992)] as to the bovine and Drosophila NPY receptors. Highly divergent sequences

within ligand-receptor families have also been reported for subtypes of amine receptors, however, no other peptide has previously been found to have receptor subtypes which display the degree of sequence divergence that exists between human Y1 (hY1-5) and bovine LCR1 (and *Drosophila* PR4). For example, the three mammalian tachykinin receptors and the two human somatostatin receptors are 58-67% and 55% identical, respectively, over the regions spanning the TM segments.

In summary, the present invention has described the cloning and identification of the human Y1-type NPY/PYY receptor. This receptor is thought to be instrumental for the ability of NPY/PYY to induce vasoconstriction as well as several behavioral effects.

As stated previously, Neuropeptide Y (NPY) is the most commonly found neurohormonal peptide in the human body. Consequently, the effects of NPY in the organism are many and varied. NPY, like many other messenger molecules, acts by stimulating specific receptor molecules on the cell surface. Previous work has shown that such receptor molecules are heterogenous and that sub-types of receptors thus exist. The receptor sub-type cloned according to the present invention is termed "Y1-receptor", and is widely believed to mediate some of the most important functions of NPY:

- (1) Vascular smooth muscle contraction - NPY is released from nerves surrounding blood vessels and is one of the most potent known pressor agents, thus increasing blood pressure in man; elevated levels of NPY have been observed in hypertensive patients;
- (2) Sedation/anxiolysis - NPY is as powerful as a benzodiazepine, e.g. Valium, in inducing anticonflict behaviors in animals; in psychiatric patients suffering from major depression, the brain levels of NPY are reduced, and anxiety symptoms in these patients are inversely related to NPY levels; and
- (3) Food intake - NPY has frequently been argued to be the most powerful stimulator of food intake and obesity ever studied in mammals; dysregulation of NPY systems have been suggested to exist in patients with eating disorders, i.e. anorexia nervosa/bulimia.

For these and other reasons, the human Y1-receptor according to the present invention has a potential pharmaceutical target; at present, no therapeutically useful drugs are known to interact with the Y1-receptor. Its isolation and cloning according to the present invention should greatly aid in screening efforts and rational drug design aiming to identify novel drugs that may either stimulate, inhibit, or block the Y1-receptor. Such drugs may thus perhaps be useful in the treatment of, e.g., hypertension, depression and/or anxiety, and eating disorders of various kinds as well as obesity. Such screening protocols are well known utilizing other receptors, and these protocols may easily be modified by those skilled in the art to incorporate the use of the Y1-receptor according to the present invention.

- - -

## EXAMPLE VI

## 15                   Production and Testing of Oligonucleotides

The oligonucleotides necessary to study the inhibition of the contractile effect of neuropeptide Y on human blood vessels were synthesized on a Biosearch Cyclone DNA Synthesizer following the manufacturer's instructions. Three oligonucleotides were prepared: (1) an antisense 18-base oligonucleotide (designated as hY1-AS) corresponding to the human Y1 receptor amino-terminus, (2) a corresponding sense oligonucleotide sequence (designated as hY1-S), and (3) a 3-base mismatched antisense oligonucleotide (designated as hY1-MM). The sequences of these three oligonucleotides were:

25           hY1-S           5' - CAACATTATT TTCCCAGG - 3'  
            hY1-AS       5' - CCTGGGAAAA TAATGTTG - 3'  
            hY1-MM       5' - CCTGAGATAA TAAGGTTG - 3'

Following deprotection with 30% ammonium hydroxide using conventional protocols, the oligonucleotides were lyophilized and redissolved in water. These oligonucleotides were then run on a 15% acrylamide gel to verify their sizes.

Subcutaneous arteries and veins from patients operated upon for non-vascular diseases were dissected in the beginning of the operation from the abdominal region and cut into cylindrical segments 2-3 mm



long. These segments were incubated in Dulbecco's Modified Essential Medium (Sigma) supplemented with streptomycin (10,000 mg/ml), penicillin (10,000 U/ml) with or without the test oligonucleotides at 1  $\mu$ M. Each incubation was conducted for 48 hours at 37° C in humidified  
5 5% carbon dioxide and 95% air.

The cylindrical segments were then mounted on two metal prongs, one of which was connected to a force displacement transducer (model FT03C) attached to a Grass Polygraph for continuous recording of the isometric tension, and the other to a displacement device. The  
10 mounted specimens were immersed in temperature controlled (37° C) tissue bathes containing a buffer of the following composition (mM): NaCl 119, NaHCO<sub>3</sub> 15, KCl 4.6, MgCl 1.2, NaH<sub>2</sub>PO<sub>4</sub> 1.2, CaCl<sub>2</sub> 1.5, and glucose 11. The solution was continuously gassed with 5% carbon dioxide in oxygen giving a pH of 7.4. A tension of 4 mN was applied to  
15 the vessel segments and they were allowed to stabilize at this level of tension for 1.5 hours. The contractile capacity of each vessel segment was examined by exposure to a potassium-rich (60 mM) buffer solution. After another 45 minutes rest period, the following known agonists were added to the vessels in cumulative doses: neuropeptide Y (Auspep,  
20 Australia), neuropeptide Y<sub>13-16</sub> (Bissendorf Biochemicals), pro<sup>34</sup>neuropeptide, noradrenaline (Sigma).

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In the human subcutaneous arteries and veins examined on day 1 without preincubation, NPY, PYY and Pro<sup>34</sup>NPY had similar contractile  
25 effects while NPY<sub>13-36</sub> had no contractile effect upon the vessels tested, thus clearly indicating that the contractions seen were mediated by a Y<sub>1</sub>-receptor.

Despite the 48 hour incubation, the vessels responded with powerful contractions to 60 mM KCl ( $3.09 \pm 0.27$  mN), with no  
30 difference between the groups receiving or not receiving the oligonucleotides describe above. The contractile responses to neuropeptide Y did not differ between the untreated group (that group receiving no oligonucleotides), the sense oligonucleotide-treated group,

or the vessels incubated with mismatched oligonucleotides either in arteries or veins as depicted in Figure 3 and the following table:

Table 1

Effect of Antisense Oligonucleotide Treatment On Potency and Maximum Contraction in Human Subcutaneous Arteries and Veins

	<u>Maximum Contraction</u>	<u>Potency</u>
<u>Human subcutaneous artery</u>		
Control (no oligonucleotide)	80.8 ± 13.7%	7.49 ± 0.38
Antisense	20.2 ± 6.8%*	7.13 ± 0.15
1 0 Sense	79.4 ± 21.7%	7.30 ± 0.19
Mismatch	88.7 ± 39.0	7.24 ± 0.26
<u>Human subcutaneous vein</u>		
Control (no oligonucleotide)	74.3 ± 7.7%	7.61 ± 0.14
Antisense	33.4 ± 5.7%*	7.26 ± 0.24
1 5 Sense	57.7 ± 9.3%	7.51 ± 0.21
Mismatch	63.9 ± 28.8%	7.78 ± 0.05

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The maximum contraction (% of potassium-induced contraction) was significantly reduced by antisense oligonucleotide treatment as shown in the above table. In this table, potency is expressed as -log concentration of agonist inducing half maximum concentration, and no significant differences were seen in the potency values between the groups (artery and vein). All values represent the mean ± SEM for 8 to 10 vessel segments, except for the mismatch value which represents 3 segments. The asterisk (\*) represents a  $p < 0.01$  between the sense and antisense data according to the paired Wilcoxon signed rank test.

As seen in Table 1, in both arteries and veins treated with Y1 receptor antisense oligonucleotide the contractile responses to NPY were markedly attenuated. This inhibition did not appear to be competitive in nature, since the potency values were not different between the groups. The responses to noradrenaline ( $10^{-9}$  -  $10^{-4}$  M) or 60 mM KCl did not differ between the groups.

As seen, after a 48 hour incubation period with the antisense oligonucleotide (1  $\mu$ M), the contractile responses to NPY were markedly

reduced. This is believed to be due to reduced numbers of NPY-Y1 receptors. This finding, in turn, indicates that the contractile effect of NPY on human resistance vessels that are likely to be active in the regulation of vascular tone and blood pressure, is mediated primarily by the cloned Y1 receptor. The selectivity of the antisense oligodeoxynucleotide molecule seems to be very high since treatment with the mismatched analogue, hY1-MM, with 3 out of 18 nucleotides mismatched, was without effect on NPY-evoked vasoconstriction. Moreover, the antisense oligonucleotide did not affect responses of the vessels to noradrenaline or high K<sup>+</sup> depolarization.

The antisense oligonucleotides described herein or deemed to be equivalents hereof, may be used in diagnostics, therapeutics and as research reagents and kits. For example, the use of the antisense oligonucleotide compounds may represent a suitable research tool for vascular pharmacology by which the functional characteristics of a number of cloned receptors may be examined. For therapeutic use, the antisense oligonucleotides according to the present invention is to be administered to an animal, especially a human, in which it is medically desired to specifically attenuate NPY-evoked vasoconstriction.

Administration of the antisense oligonucleotides according to the present invention may be by any acceptable means, however, it is most preferred that the administration take place intravenously into a blood vessel, either artery or vein, so as to deliver the oligonucleotide directly to the site of NPY receptors. Use of recognized pharmacologically acceptable carriers may also be preferred as carriers, diluents, buffers and other functional classes well within the purview of those skilled in the formulation arts. The exact dosages of antisense oligonucleotides provided to a mammal to attenuate the NPY-evoked response in the mammal's blood vessels may vary across a broad range, however, such dosages should be limited to that range which is sufficient to bring about the desired degree of attenuation based upon the method of administration, the urgency by which such attenuation is desired, the weight of the mammal, and the amount of the oligonucleotide in the total bolus of medication administered. Such

variables are well within the purview of those skilled in the compounding and administration arts, and thus uniqueness for the use of antisense oligonucleotides to the human NPY receptor is not to be predicated upon any specific amount of oligonucleotide being administered to the mammal in which vasoconstriction inhibition is desired.

A list of the nucleic acid and amino acid sequences which comprise the present invention follows:

#### SEQUENCE LISTING

##### 1 0 (1) GENERAL INFORMATION:

(i) APPLICANT: Claes R. Wahlestedt

(iii) NUMBER OF SEQUENCES: 6

##### (2) INFORMATION FOR SEQ ID NO:1:

##### (i) SEQUENCE CHARACTERISTICS:

1 5 (A) LENGTH: 1534 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

##### 2 0 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCTTCTTTTAA	TGAAGCAGGA	GCGAAAAAGA	CAAATTCCAA	AGAGGATTGT	50
TCAGTTCAAG	GGAATGAAGA	ATTCAGAATA	ATTTTGGTAA	ATGGATTCCA	100
ATATGGGGAA	TAAGAATAAG	CTGAACAGTT	GACCTGCTTT	GAAGAAACAT	150
ACTGTCCATT	TGTCTAAAAT	AATCTATAAC	AACCAAACCA	ATCAAA	196
2 5 ATG AAT TCA ACA TTA TTT TCC CAG GTT GAA AAT CAT TCA	235				
GTC CAC TCT AAT TTC TCA GAG AAG AAT GCC CAG CTT CTG	274				
GCT TTT GAA AAT GAT GAT TGT CAT CTG CCC TTG GCC ATG	313				
ATA TTT ACC TTA GCT CTT GCT TAT GGA GCT GTG ATC ATT	352				
CTT GTC TCT GGA AAC CTG GCC TTG ATC ATA ATC ATC TTG	391				
3 0 AAA CAA AAG GAG ATG AGA AAT GTT ACC AAC ATC CTG ATT	430				
GTG AAC CTT TCC TTC TCA GAC TTG CTT GTT GCC ATC ATG	469				
TGT CTC CCC TTT ACA TTT GTC TAC ACA TTA ATG GAC CAC	508				
TGG GTC TTT GGT GAG GCG ATG TGT AAG TTG AAT CCT TTT	547				
GTG CAA TGT GTT TCA ATC ACT GTG TCC ATT TTC TCT CTG	586				
3 5 GTT CTC ATT GCT GTG GAA CGA CAT CAG CTG ATA ATC AAC	625				

CCT CGA GGG TGG AGA CCA AAT AAT AGA CAT GCT TAT GTA 664  
 GGT ATT GCT GTG ATT TGG GTC CTT GCT GTG GCT TCT TCT 703  
 TTG CCT TTC CTG ATC TAC CAA GTA ATG ACT GAT GAG CCG 742  
 TTC CAA AAT GTA ACA CTT GAT GCG TAC AAA GAC AAA TAC 781  
 5 GTG TGC TTT GAT CAA TTT CCA TCG GAC TCT CAT AGG TTG 820  
 TCT TAT ACC ACT CTC CTC TTG GTG CTG CAG TAT TTT GGT 859  
 CCA CTT TGT TTT ATA TTT ATT TGC TAC TTC AAG ATA TAT 898  
 ATA CGC CTA AAA AGG AGA AAC AAC ATG ATG GAC AAG ATG 937  
 AGA GAC AAT AAG TAC AGG TCC AGT GAA ACC AAA AGA ATC 976  
 10 AAT ATC ATG CTG CTC TCC ATT GTG GTA GCA TTT GCA GTC 1015  
 TGC TGG CTC CCT CTT ACC ATC TTT AAC ACT GTG TTT GAT 1054  
 TGG AAT CAT CAG ATC ATT GCT ACC TGC AAC CAC AAT CTG 1093  
 TTA TTC CTG CTC TGC CAC CTC ACA GCA ATG ATA TCC ACT 1132  
 TGT GTC AAC CCC ATA TTT TAT GGG TTC CTG AAC AAA AAC 1171  
 15 TTC CAG AGA GAC TTG CAG TTC TTC TTC AAC TTT TGT GAT 1210  
 TTC CGG TCT CGG GAT GAT GAT TAT GAA ACA ATA GCC ATG 1249  
 TCC ACG ATG CAC ACA GAT GTT TCC AAA ACT TCT TTG AAG 1288  
 CAA GCA AGC CCA GTC GCA TTT AAA AAA ATC AAC AAC AAT 1327  
 GAT GAT AAT GAA AAA ATC TGA AAC TAC TTA TAG CCT ATG 1366  
 20 GTC CCG GAT GAC ATC TGT TTA AAA ACA AGC ACA ACC TGC 1405  
 AAC ATA CTT TGA TTA CCT GTT CTC CCA AGG AAT GGG GTT 1444  
 GAA ATC ATT TGA AAA TGA CTA AGA TTT TCT TGT CTT GCT 1483  
 TTT TAC AGT TTT GAC CAG ACA TCT TTG AAG TGC TTT TTG 1522  
 TGA ATT TAC CAG 1534

2.5 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1338 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 3.0 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATG AAT TCA ACA TTA TTT TCC CAG GTT GAA AAT CAT TCA 39  
 GTC CAC TCT AAT TTC TCA GAG AAG AAT GCC CAG CTT CTG 78  
 3.5 GCT TTT GAA AAT GAT GAT TGT CAT CTG CCC TTG GCC ATG 117

	ATA TTT ACC TTA GCT CTT GCT TAT GGA GCT GTG ATC ATT	156
	CTT GTC TCT GGA AAC CTG GCC TTG ATC ATA ATC ATC TTG	195
	AAA CAA AAG GAG ATG AGA AAT GTT ACC AAC ATC CTG ATT	234
	GTG AAC CTT TCC TTC TCA GAC TTG CTT GTT GCC ATC ATG	273
5	TGT CTC CCC TTT ACA TTT GTC TAC ACA TTA ATG GAC CAC	312
	TGG GTC TTT GGT GAG GOG ATG TGT AAG TTG AAT CCT TTT	351
	GTG CAA TGT GTT TCA ATC ACT GTG TCC ATT TTC TCT CTG	390
	GTT CTC ATT GCT GTG GAA CGA CAT CAG CTG ATA ATC AAC	429
	CCT CGA GGG TGG AGA CCA AAT AAT AGA CAT GCT TAT GTA	468
10	GGT ATT GCT GTG ATT TGG GTC CTT GCT GTG GCT TCT TCT	507
	TTG CCT TTC CTG ATC TAC CAA GTA ATG ACT GAT GAG CCG	546
	TTC CAA AAT GTA ACA CTT GAT GOG TAC AAA GAC AAA TAC	585
	GTG TGC TTT GAT CAA TTT CCA TCG GAC TCT CAT AGG TTG	624
	TCT TAT ACC ACT CTC CTC TTG GTG CTG CAG TAT TTT GGT	663
15	CCA CTT TGT TTT ATA TTT ATT TGC TAC TTC AAG ATA TAT	702
	ATA CGC CTA AAA AGG AGA AAC AAC ATG ATG GAC AAG ATG	741
	AGA GAC AAT AAG TAC AGG TCC AGT GAA ACC AAA AGA ATC	780
	AAT ATC ATG CTG CTC TCC ATT GTG GTA GCA TTT GCA GTC	819
	TGC TGG CTC CCT CTT ACC ATC TTT AAC ACT GTG TTT GAT	858
20	TGG AAT CAT CAG ATC ATT GCT ACC TGC AAC CAC AAT CTG	897
	TTA TTC CTG CTC TGC CAC CTC ACA GCA ATG ATA TCC ACT	936
	TGT GTC AAC CCC ATA TTT TAT GGG TTC CTG AAC AAA AAC	975
	TTC CAG AGA GAC TTG CAG TTC TTC TTC AAC TTT TGT GAT	1014
	TTC CGG TCT CGG GAT GAT GAT TAT GAA ACA ATA GCC ATG	1053
25	TCC ACG ATG CAC ACA GAT GTT TCC AAA ACT TCT TTG AAG	1092
	CAA GCA AGC CCA GTC GCA TTT AAA AAA ATC AAC AAC AAT	1131
	GAT GAT AAT GAA AAA ATC TGA AAC TAC TTA TAG CCT ATG	1170
	GTC CCG GAT GAC ATC TGT TTA AAA ACA AGC ACA ACC TGC	1209
	AAC ATA CTT TGA TTA CCT GTT CTC CCA AGG AAT GGG GTT	1248
30	GAA ATC ATT TGA AAA TGA CTA AGA TTT TCT TGT CTT GCT	1287
	TTT TAC AGT TTT GAC CAG ACA TCT TTG AAG TGC TTT TTG	1326
	TGA ATT TAC CAG	1338

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 384 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

	Met	Asn	Ser	Thr	Leu	Phe	Ser	Gln	Val	Glu	Asn	His	Ser	Val	His	
					5					10					15	
10	Ser	Asn	Phe	Ser	Glu	Lys	Asn	Ala	Gln	Leu	Leu	Ala	Phe	Glu	Asn	
					20					25					30	
	Asp	Asp	Cys	His	Leu	Pro	Leu	Ala	Met	Ile	Phe	Thr	Leu	Ala	Leu	
					35					40					45	
	Ala	Tyr	Gly	Ala	Val	Ile	Ile	Leu	Gly	Val	Ser	Gly	Asn	Leu	Ala	
					50					55					60	
15	Leu	Ile	Ile	Ile	Ile	Leu	Lys	Gln	Lys	Glu	Met	Arg	Asn	Val	Thr	
					65					70					75	
	Asn	Ile	Leu	Ile	Val	Asn	Leu	Ser	Phe	Ser	Asp	Leu	Leu	Val	Ala	
					80					85					90	
	Ile	Met	Cys	Leu	Pro	Phe	Thr	Phe	Val	Tyr	Thr	Leu	Met	Asp	His	
20					95					100					105	
	Trp	Val	Phe	Gly	Glu	Ala	Met	Cys	Lys	Leu	Asn	Pro	Phe	Val	Gln	
					110					115					120	
	Cys	Val	Ser	Ile	Thr	Val	Ser	Ile	Phe	Ser	Leu	Val	Leu	Ile	Ala	
					125					130					135	
25	Val	Glu	Arg	His	Gln	Leu	Ile	Ile	Asn	Pro	Arg	Gly	Trp	Arg	Pro	
					140					145					150	
	Asn	Asn	Arg	His	Ala	Tyr	Val	Gly	Ile	Ala	Val	Ile	Trp	Val	Leu	
					155					160					165	
	Ala	Val	Ala	Ser	Ser	Leu	Pro	Phe	Leu	Ile	Tyr	Gln	Val	Met	Thr	
30					170					175					180	
	Asp	Glu	Pro	Phe	Gln	Asn	Val	Thr	Leu	Asp	Ala	Tyr	Lys	Asp	Lys	
					185					190					195	
	Tyr	Val	Cys	Phe	Asp	Gln	Phe	Pro	Ser	Asp	Ser	His	Arg	Leu	Ser	
					200					205					210	
35	Tyr	Thr	Thr	Leu	Leu	Leu	Val	Leu	Gln	Tyr	Phe	Gly	Pro	Leu	Cys	
					215					220					225	
	Phe	Ile	Phe	Ile	Cys	Tyr	Phe	Lys	Ile	Tyr	Ile	Arg	Leu	Lys	Arg	
					230					235					240	
	Arg	Asn	Asn	Met	Met	Asp	Lys	Met	Arg	Asp	Asn	Lys	Tyr	Arg	Ser	
40					245					250					255	
	Ser	Glu	Thr	Lys	Arg	Ile	Asn	Ile	Met	Leu	Leu	Ser	Ile	Val	Val	
					260					265					270	

Ala Phe Ala Val Cys Trp Leu Pro Leu Thr Ile Phe Asn Thr Val  
                                   275                                  280                                  285  
 Phe Asp Trp Asn His Gln Ile Ile Ala Thr Cys Asn His Asn Leu  
                                   290                                  295                                  300  
 5 Leu Phe Leu Leu Cys His Leu Thr Ala Met Ile Ser Thr Cys Val  
                                   305                                  310                                  315  
 Asn Pro Ile Phe Tyr Gly Phe Leu Asn Lys Asn Phe Gln Arg Asp  
                                   320                                  325                                  330  
 10 Leu Gln Phe Phe Phe Asn Phe Cys Asp Phe Arg Ser Arg Asp Asp  
                                   335                                  340                                  345  
 Asp Tyr Glu Thr Ile Ala Met Ser Thr Met His Thr Asp Val Ser  
                                   350                                  355                                  360  
 Lys Thr Ser Leu Lys Gln Ala Ser Pro Val Ala Phe Lys Lys Ile  
                                   365                                  370                                  375  
 15 Asn Asn Asn Asp Asp Asn Glu Lys Ile  
                                   380

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 18 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

25 CAACATTATT TTCCCAGG 18

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 18 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCTGGGAAAA TAATGTTG 18

## 35 (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single



(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCTGAGATAA TAAGGTTG 18

5        Thus while I have illustrated and described the preferred  
embodiment of my invention, it is to be understood that this invention  
is capable of variation and modification, and I therefore do not wish to  
be limited to the precise terms set forth, but desire to avail myself of  
such changes and alterations which may be made for adapting the  
10 invention to various usages and conditions. Such variations and  
modifications, for example, would include the substitution of  
structurally similar nucleic and amino acid sequences which function to  
yield substantially similar activities to those specifically described  
above. Thus, changes in sequence by the substitution, deletion,  
15 insertion or addition of nucleic acids (in the DNA sequences), or  
substitution of completely different antisense sequences which do not  
substantially alter the function of those sequences specifically  
described above, are deemed to be within the scope of the present  
invention. Accordingly, such changes and alterations are properly  
20 intended to be within the full range of equivalents, and therefore within  
the purview of the following claims.

Having thus described my invention and the manner and a process  
of making and using it in such full, clear, concise and exact terms so as  
to enable any person skilled in the art to which it pertains, or with  
25 which it is most nearly connected, to make and use the same;

I claim:

1. An antisense oligonucleotide sequence, corresponding to the amino-terminus of the human Y1 receptor, that is 5' - CCTGGGAAAA TAATGTTG - 3', said sequence being further characterized as having the specific pharmacologic action of attenuating neuropeptide Y-evoked vasoconstriction in human arteries and veins.

2. A method for attenuating neuropeptide Y-evoked contractile response in a mammalian blood vessel which comprises contacting human Y1 receptors affecting the contractile response with an antisense oligonucleotide to the receptor in an amount sufficient to bring about an attenuation of the neuropeptide Y-evoked response.

3. A method according to Claim 2 in which the antisense oligonucleotide is 5' - CCTGGGAAAA TAATGTTG - 3'.

4. A cDNA for the genetic encoding of the human neuropeptide Y/peptide YY Y-1 receptor which is

CCTTCTTTAA TGAAGCAGGA GCGAAAAAGA CAAATTCCAA AGAGGATTGT	50
TCAGTTCAAG GGAATGAAGA ATTCAGAATA ATTTTGGTAA ATGGATTCCA	100
ATATGGGGAA TAAGAATAAG CTGAACAGTT GACCTGCTTT GAAGAAACAT	150
ACTGTCCATT TGTCTAAAAT AATCTATAAC AACCAAACCA ATCAAA	196
ATG AAT TCA ACA TTA TTT TCC CAG GTT GAA AAT CAT TCA	235
GTC CAC TCT AAT TTC TCA GAG AAG AAT GCC CAG CTT CTG	274
GCT TTT GAA AAT GAT GAT TGT CAT CTG CCC TTG GCC ATG	313
ATA TTT ACC TTA GCT CTT GCT TAT GGA GCT GTG ATC ATT	352
CTT GTC TCT GGA AAC CTG GCC TTG ATC ATA ATC ATC TTG	391
AAA CAA AAG GAG ATG AGA AAT GTT ACC AAC ATC CTG ATT	430
GTG AAC CTT TCC TTC TCA GAC TTG CTT GTT GCC ATC ATG	469
TGT CTC CCC TTT ACA TTT GTC TAC ACA TTA ATG GAC CAC	508
TGG GTC TTT GGT GAG GCG ATG TGT AAG TTG AAT CCT TTT	547
GTG CAA TGT GTT TCA ATC ACT GTG TCC ATT TTC TCT CTG	586
GTT CTC ATT GCT GTG GAA CGA CAT CAG CTG ATA ATC AAC	625
CCT CGA GGG TGG AGA CCA AAT AAT AGA CAT GCT TAT GTA	664
GGT ATT GCT GTG ATT TGG GTC CTT GCT GTG GCT TCT TCT	703
TTG CCT TTC CTG ATC TAC CAA GTA ATG ACT GAT GAG CCG	742
TTC CAA AAT GTA ACA CTT GAT GCG TAC AAA GAC AAA TAC	781

GTG TGC TTT GAT CAA TTT CCA TCG GAC TCT CAT AGG TTG 820  
 TCT TAT ACC ACT CTC CTC TTG GTG CTG CAG TAT TTT GGT 859  
 CCA CTT TGT TTT ATA TTT ATT TGC TAC TTC AAG ATA TAT 898  
 ATA CGC CTA AAA AGG AGA AAC AAC ATG ATG GAC AAG ATG 937  
 AGA GAC AAT AAG TAC AGG TCC AGT GAA ACC AAA AGA ATC 976  
 AAT ATC ATG CTG CTC TCC ATT GTG GTA GCA TTT GCA GTC 1015  
 TGC TGG CTC CCT CTT ACC ATC TTT AAC ACT GTG TTT GAT 1054  
 TGG AAT CAT CAG ATC ATT GCT ACC TGC AAC CAC AAT CTG 1093  
 TTA TTC CTG CTC TGC CAC CTC ACA GCA ATG ATA TCC ACT 1132  
 TGT GTC AAC CCC ATA TTT TAT GGG TTC CTG AAC AAA AAC 1171  
 TTC CAG AGA GAC TTG CAG TTC TTC TTC AAC TTT TGT GAT 1210  
 TTC CGG TCT CGG GAT GAT GAT TAT GAA ACA ATA GCC ATG 1249  
 TCC ACG ATG CAC ACA GAT GTT TCC AAA ACT TCT TTG AAG 1288  
 CAA GCA AGC CCA GTC GCA TTT AAA AAA ATC AAC AAC AAT 1327  
 GAT GAT AAT GAA AAA ATC TGA AAC TAC TTA TAG CCT ATG 1366  
 GTC CCG GAT GAC ATC TGT TTA AAA ACA AGC ACA ACC TGC 1405  
 AAC ATA CTT TGA TTA CCT GTT CTC CCA AGG AAT GGG GTT 1444  
 GAA ATC ATT TGA AAA TGA CTA AGA TTT TCT TGT CTT GCT 1483  
 TTT TAC AGT TTT GAC CAG ACA TCT TTG AAG TGC TTT TTG 1522  
 TGA ATT TAC CAG 1534.

5. A cDNA according to Claim 4 which is the structural gene for human neuropeptide Y/peptide YY Y-1 receptor and which is:

ATG AAT TCA ACA TTA TTT TCC CAG GTT GAA AAT CAT TCA 39  
 GTC CAC TCT AAT TTC TCA GAG AAG AAT GCC CAG CTT CTG 78  
 GCT TTT GAA AAT GAT GAT TGT CAT CTG CCC TTG GCC ATG 117  
 ATA TTT ACC TTA GCT CTT GCT TAT GGA GCT GTG ATC ATT 156  
 CTT GTC TCT GGA AAC CTG GCC TTG ATC ATA ATC ATC TTG 195  
 AAA CAA AAG GAG ATG AGA AAT GTT ACC AAC ATC CTG ATT 234  
 GTG AAC CTT TCC TTC TCA GAC TTG CTT GTT GCC ATC ATG 273  
 TGT CTC CCC TTT ACA TTT GTC TAC ACA TTA ATG GAC CAC 312  
 TGG GTC TTT GGT GAG GCG ATG TGT AAG TTG AAT CCT TTT 351  
 GTG CAA TGT GTT TCA ATC ACT GTG TCC ATT TTC TCT CTG 390  
 GTT CTC ATT GCT GTG GAA CGA CAT CAG CTG ATA ATC AAC 429  
 CCT CGA GGG TGG AGA CCA AAT AAT AGA CAT GCT TAT GTA 468

GGT ATT GCT GTG ATT TGG GTC CTT GCT GTG GCT TCT TCT 507  
TTG CCT TTC CTG ATC TAC CAA GTA ATG ACT GAT GAG CCG 546  
TTC CAA AAT GTA ACA CTT GAT GCG TAC AAA GAC AAA TAC 585  
GTG TGC TTT GAT CAA TTT CCA TCG GAC TCT CAT AGG TTG 624  
TCT TAT ACC ACT CTC CTC TTG GTG CTG CAG TAT TTT GGT 663  
CCA CTT TGT TTT ATA TTT ATT TGC TAC TTC AAG ATA TAT 702  
ATA CGC CTA AAA AGG AGA AAC AAC ATG ATG GAC AAG ATG 741  
AGA GAC AAT AAG TAC AGG TCC AGT GAA ACC AAA AGA ATC 780  
AAT ATC ATG CTG CTC TCC ATT GTG GTA GCA TTT GCA GTC 819  
TGC TGG CTC CCT CTT ACC ATC TTT AAC ACT GTG TTT GAT 858  
TGG AAT CAT CAG ATC ATT GCT ACC TGC AAC CAC AAT CTG 897  
TTA TTC CTG CTC TGC CAC CTC ACA GCA ATG ATA TCC ACT 936  
TGT GTC AAC CCC ATA TTT TAT GGG TTC CTG AAC AAA AAC 975  
TTC CAG AGA GAC TTG CAG TTC TTC TTC AAC TTT TGT GAT 1014  
TTC CGG TCT CGG GAT GAT GAT TAT GAA ACA ATA GCC ATG 1053  
TCC ACG ATG CAC ACA GAT GTT TCC AAA ACT TCT TTG AAG 1092  
CAA GCA AGC CCA GTC GCA TTT AAA AAA ATC AAC AAC AAT 1131  
GAT GAT AAT GAA AAA ATC TGA AAC TAC TTA TAG CCT ATG 1170  
GTC CCG GAT GAC ATC TGT TTA AAA ACA AGC ACA ACC TGC 1209  
AAC ATA CTT TGA TTA CCT GTT CTC CCA AGG AAT GGG GTT 1248  
GAA ATC ATT TGA AAA TGA CTA AGA TTT TCT TGT CTT GCT 1287  
TTT TAC AGT TTT GAC CAG ACA TCT TTG AAG TGC TTT TTG 1326  
TGA ATT TAC CAG 1338

#### 6. The isolated peptide

Met	Asn	Ser	Thr	Leu	Phe	Ser	Gln	Val	Glu	Asn	His	Ser	Val	His
				5					10					15
Ser	Asn	Phe	Ser	Glu	Lys	Asn	Ala	Gln	Leu	Leu	Ala	Phe	Glu	Asn
				20					25					30
Asp	Asp	Cys	His	Leu	Pro	Leu	Ala	Met	Ile	Phe	Thr	Leu	Ala	Leu
				35					40					45
Ala	Tyr	Gly	Ala	Val	Ile	Ile	Leu	Gly	Val	Ser	Gly	Asn	Leu	Ala
				50					55					60
Leu	Ile	Ile	Ile	Ile	Leu	Lys	Gln	Lys	Glu	Met	Arg	Asn	Val	Thr
				65					70					75
Asn	Ile	Leu	Ile	Val	Asn	Leu	Ser	Phe	Ser	Asp	Leu	Leu	Val	Ala
				80					85					90

Ile Met Cys Leu Pro Phe Thr Phe Val Tyr Thr Leu Met Asp His		
	95	100
Trp Val Phe Gly Glu Ala Met Cys Lys Leu Asn Pro Phe Val Gln		105
	110	115
Cys Val Ser Ile Thr Val Ser Ile Phe Ser Leu Val Leu Ile Ala		120
	125	130
Val Glu Arg His Gln Leu Ile Ile Asn Pro Arg Gly Trp Arg Pro		135
	140	145
Asn Asn Arg His Ala Tyr Val Gly Ile Ala Val Ile Trp Val Leu		150
	155	160
Ala Val Ala Ser Ser Leu Pro Phe Leu Ile Tyr Gln Val Met Thr		165
	170	175
Asp Glu Pro Phe Gln Asn Val Thr Leu Asp Ala Tyr Lys Asp Lys		180
	185	190
Tyr Val Cys Phe Asp Gln Phe Pro Ser Asp Ser His Arg Leu Ser		195
	200	205
Tyr Thr Thr Leu Leu Leu Val Leu Gln Tyr Phe Gly Pro Leu Cys		210
	215	220
Phe Ile Phe Ile Cys Tyr Phe Lys Ile Tyr Ile Arg Leu Lys Arg		225
	230	235
Arg Asn Asn Met Met Asp Lys Met Arg Asp Asn Lys Tyr Arg Ser		240
	245	250
Ser Glu Thr Lys Arg Ile Asn Ile Met Leu Leu Ser Ile Val Val		255
	260	265
Ala Phe Ala Val Cys Trp Leu Pro Leu Thr Ile Phe Asn Thr Val		270
	275	280
Phe Asp Trp Asn His Gln Ile Ile Ala Thr Cys Asn His Asn Leu		285
	290	295
Leu Phe Leu Leu Cys His Leu Thr Ala Met Ile Ser Thr Cys Val		300
	305	310
Asn Pro Ile Phe Tyr Gly Phe Leu Asn Lys Asn Phe Gln Arg Asp		315
	320	325
Leu Gln Phe Phe Phe Asn Phe Cys Asp Phe Arg Ser Arg Asp Asp		330
	335	340
Asp Tyr Glu Thr Ile Ala Met Ser Thr Met His Thr Asp Val Ser		345
	350	355
Lys Thr Ser Leu Lys Gln Ala Ser Pro Val Ala Phe Lys Lys Ile		360
	365	370
Asn Asn Asn Asp Asp Asn Glu Lys Ile		375
	380	

7. A method for screening compounds for the treatment of a condition brought about by other than normal clinical amounts of neuropeptide Y in a patient which comprises bringing said compound in

contact with an isolated human Y1-receptor peptide, or a fragment thereof having Neuropeptide Y activity, having the amino acid sequence:

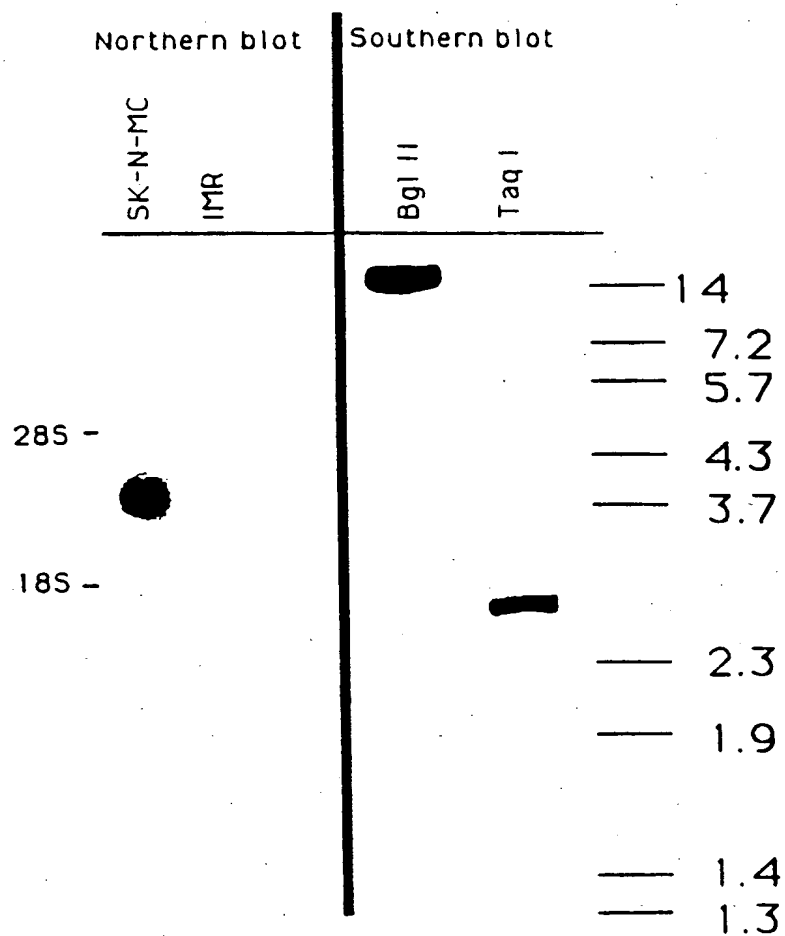
Met	Asn	Ser	Thr	Leu	Phe	Ser	Gln	Val	Glu	Asn	His	Ser	Val	His
				5					10					15
Ser	Asn	Phe	Ser	Glu	Lys	Asn	Ala	Gln	Leu	Leu	Ala	Phe	Glu	Asn
				20					25					30
Asp	Asp	Cys	His	Leu	Pro	Leu	Ala	Met	Ile	Phe	Thr	Leu	Ala	Leu
				35					40					45
Ala	Tyr	Gly	Ala	Val	Ile	Ile	Leu	Gly	Val	Ser	Gly	Asn	Leu	Ala
				50					55					60
Leu	Ile	Ile	Ile	Ile	Leu	Lys	Gln	Lys	Glu	Met	Arg	Asn	Val	Thr
				65					70					75
Asn	Ile	Leu	Ile	Val	Asn	Leu	Ser	Phe	Ser	Asp	Leu	Leu	Val	Ala
				80					85					90
Ile	Met	Cys	Leu	Pro	Phe	Thr	Phe	Val	Tyr	Thr	Leu	Met	Asp	His
				95					100					105
Trp	Val	Phe	Gly	Glu	Ala	Met	Cys	Lys	Leu	Asn	Pro	Phe	Val	Gln
				110					115					120
Cys	Val	Ser	Ile	Thr	Val	Ser	Ile	Phe	Ser	Leu	Val	Leu	Ile	Ala
				125					130					135
Val	Glu	Arg	His	Gln	Leu	Ile	Ile	Asn	Pro	Arg	Gly	Trp	Arg	Pro
				140					145					150
Asn	Asn	Arg	His	Ala	Tyr	Val	Gly	Ile	Ala	Val	Ile	Trp	Val	Leu
				155					160					165
Ala	Val	Ala	Ser	Ser	Leu	Pro	Phe	Leu	Ile	Tyr	Gln	Val	Met	Thr
				170					175					180
Asp	Glu	Pro	Phe	Gln	Asn	Val	Thr	Leu	Asp	Ala	Tyr	Lys	Asp	Lys
				185					190					195
Tyr	Val	Cys	Phe	Asp	Gln	Phe	Pro	Ser	Asp	Ser	His	Arg	Leu	Ser
				200					205					210
Tyr	Thr	Thr	Leu	Leu	Leu	Val	Leu	Gln	Tyr	Phe	Gly	Pro	Leu	Cys
				215					220					225
Phe	Ile	Phe	Ile	Cys	Tyr	Phe	Lys	Ile	Tyr	Ile	Arg	Leu	Lys	Arg
				230					235					240
Arg	Asn	Asn	Met	Met	Asp	Lys	Met	Arg	Asp	Asn	Lys	Tyr	Arg	Ser
				245					250					255
Ser	Glu	Thr	Lys	Arg	Ile	Asn	Ile	Met	Leu	Leu	Ser	Ile	Val	Val
				260					265					270
Ala	Phe	Ala	Val	Cys	Trp	Leu	Pro	Leu	Thr	Ile	Phe	Asn	Thr	Val
				275					280					285
Phe	Asp	Trp	Asn	His	Gln	Ile	Ile	Ala	Thr	Cys	Asn	His	Asn	Leu
				290					295					300

Leu Phe Leu Leu Cys His Leu Thr Ala Met Ile Ser Thr Cys Val		
	305	310
Asn Pro Ile Phe Tyr Gly Phe Leu Asn Lys Asn Phe Gln Arg Asp		
	320	325
Leu Gln Phe Phe Phe Asn Phe Cys Asp Phe Arg Ser Arg Asp Asp		
	335	340
Asp Tyr Glu Thr Ile Ala Met Ser Thr Met His Thr Asp Val Ser		
	350	355
Lys Thr Ser Leu Lys Gln Ala Ser Pro Val Ala Phe Lys Lys Ile		
	365	370
Asn Asn Asn Asp Asp Asn Glu Lys Ile		
	380	

and determining whether said compound stimulates, inhibits or blocks the human Y1-receptor following conventional screening protocols.

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FIGURE 1

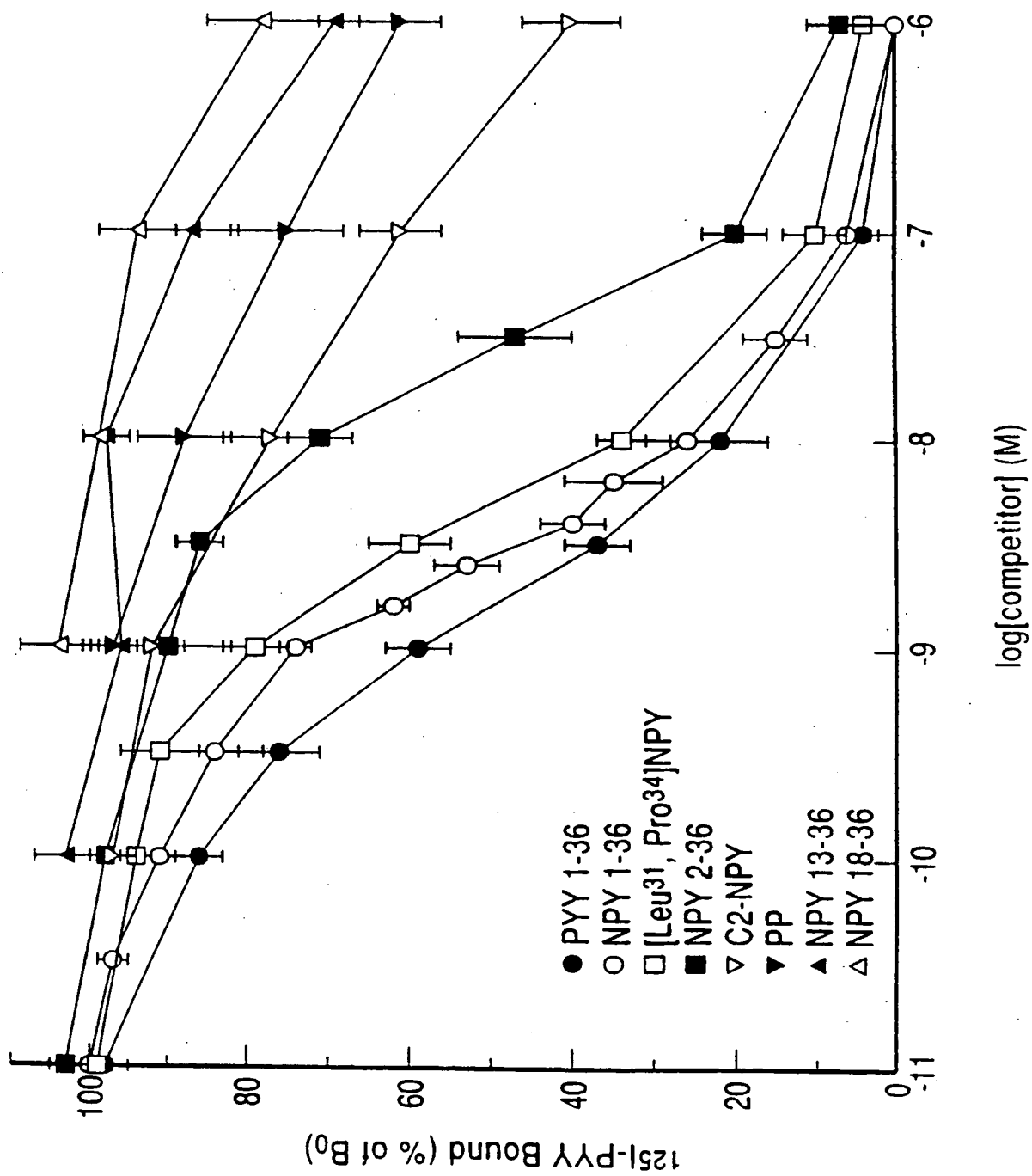


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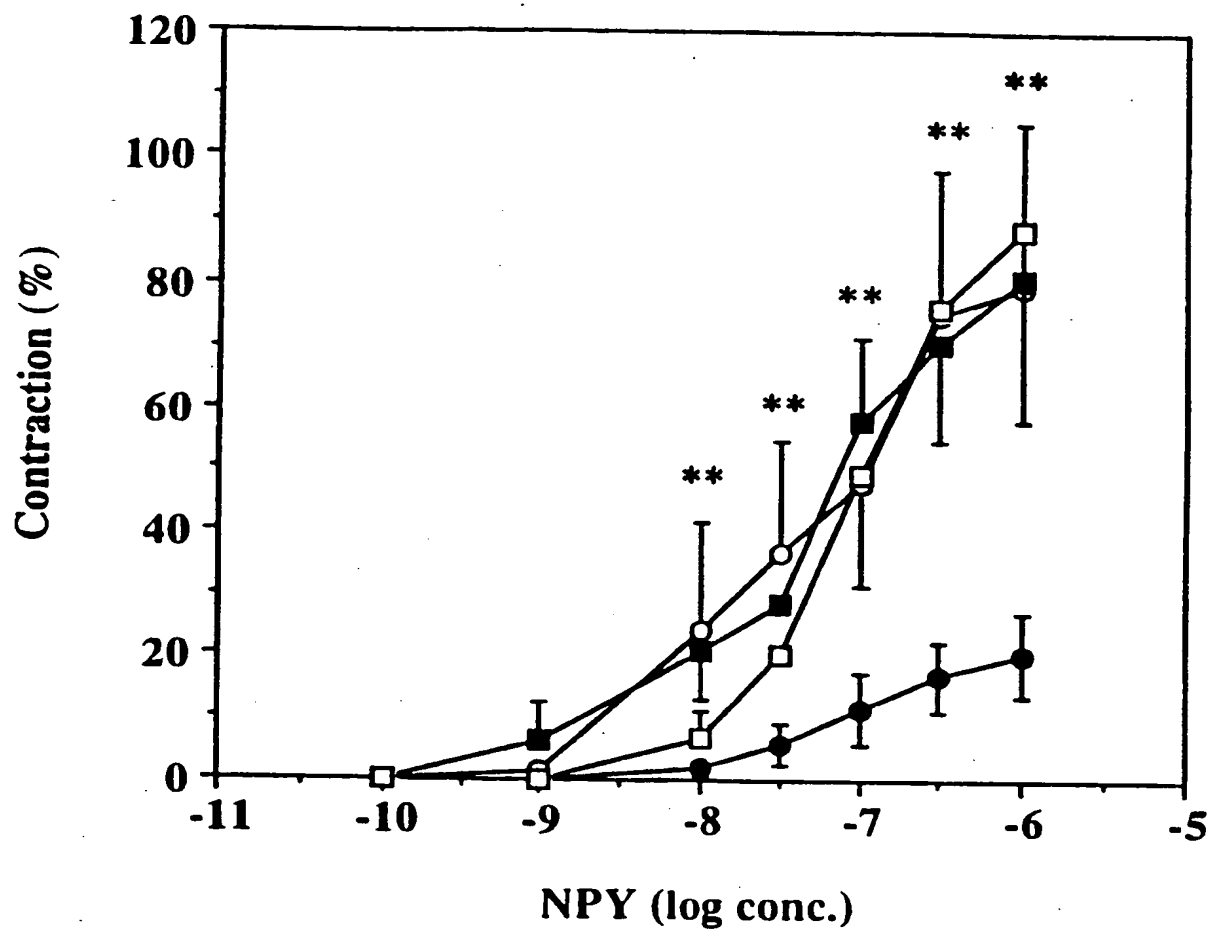
FIGURE 2



SUBSTITUTE SHEET

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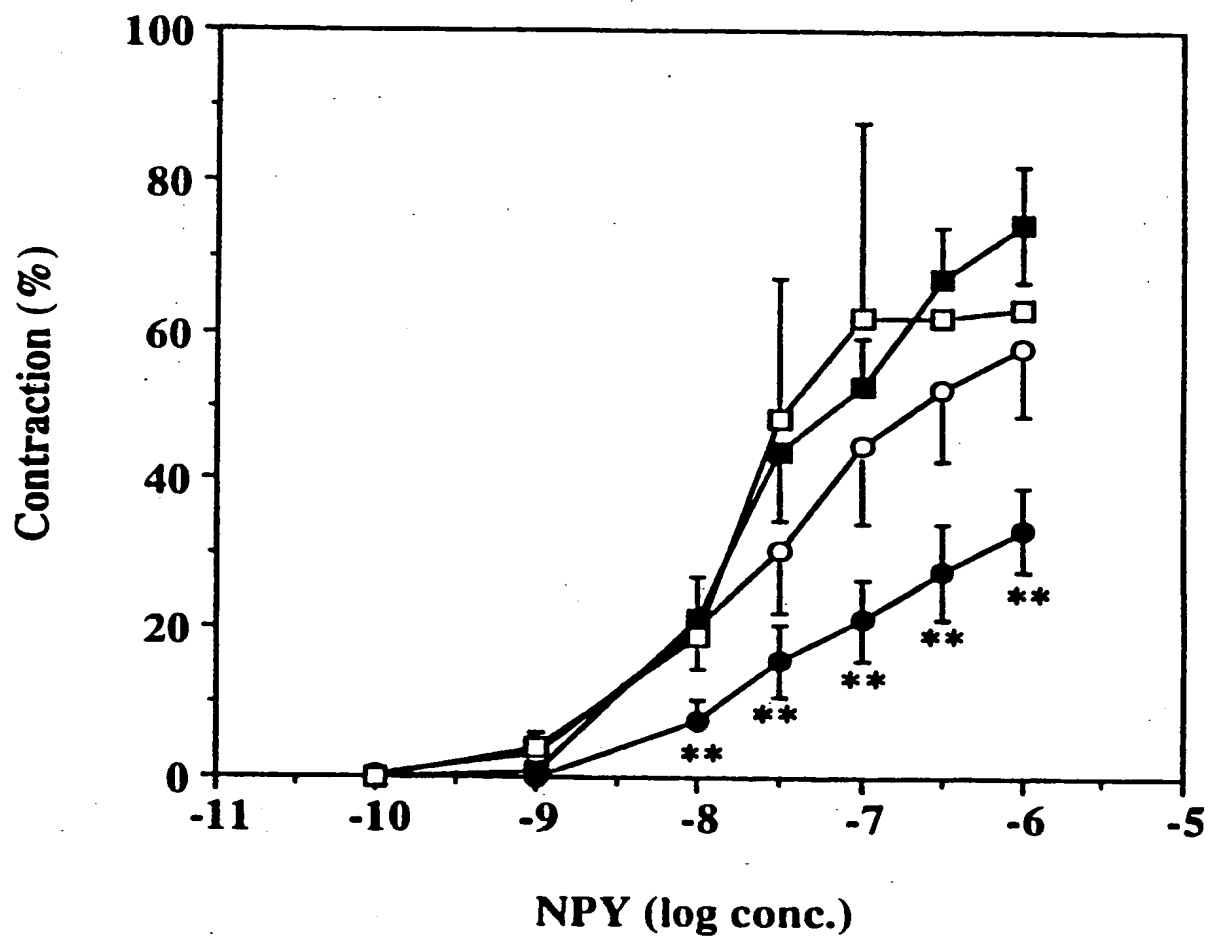
FIGURE 3



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FIGURE 4



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/05039**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : C07H 21/04; C12N 1/21, 5/00; C12P 21/00

US CL : 536/23.1; 530/350; 514/12; 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 530/350; 514/12; 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline, Dialog

search terms: neuropeptide Y, peptide YY, receptor

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	The Journal of Biological Chemistry, Volume 267, No. 1, issued 05 January 1992, X. Li et al, "Cloning, Functional Expression, and Developmental Regulation of a Neuropeptide Y Receptor from Drosophila Melanogaster", pages 9-12, see abstract.	1-8
Y	European Journal of Pharmacology, Volume 204, issued 1991, H. N. Doods et al, "Different Neuropeptide Y Receptor Subtypes in Rat and Rabbit Vas Deferens", pages 101-103, see abstract.	1-8

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*A*	Special categories of cited documents:	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*E*	document defining the general state of the art which is not considered to be part of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L*	earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O*	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
*P*	document referring to an oral disclosure, use, exhibition or other means		
*P*	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

20 AUGUST 1993

Date of mailing of the international search report

SEP 13 1993

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Authorized officer

SALLY P. TENG

Facsimile No. NOT APPLICABLE

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US93/05039

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Molecular Pharmacolog, Volume 40, issued 1991, J. Rimland et al, "Sequence and Expression of a Neuropeptide Y Receptor cDNA", pages 869-875, see abstract.	1-8
Y	Annals of the New York Academy of Sciences, Volume 611, issued 01 December 1990, C. Wahlestedt et al, "Central and Peripheral Significance of Neuropeptide Y and its Related Peptides", pages 7-26, see pages 11-18.	1-8